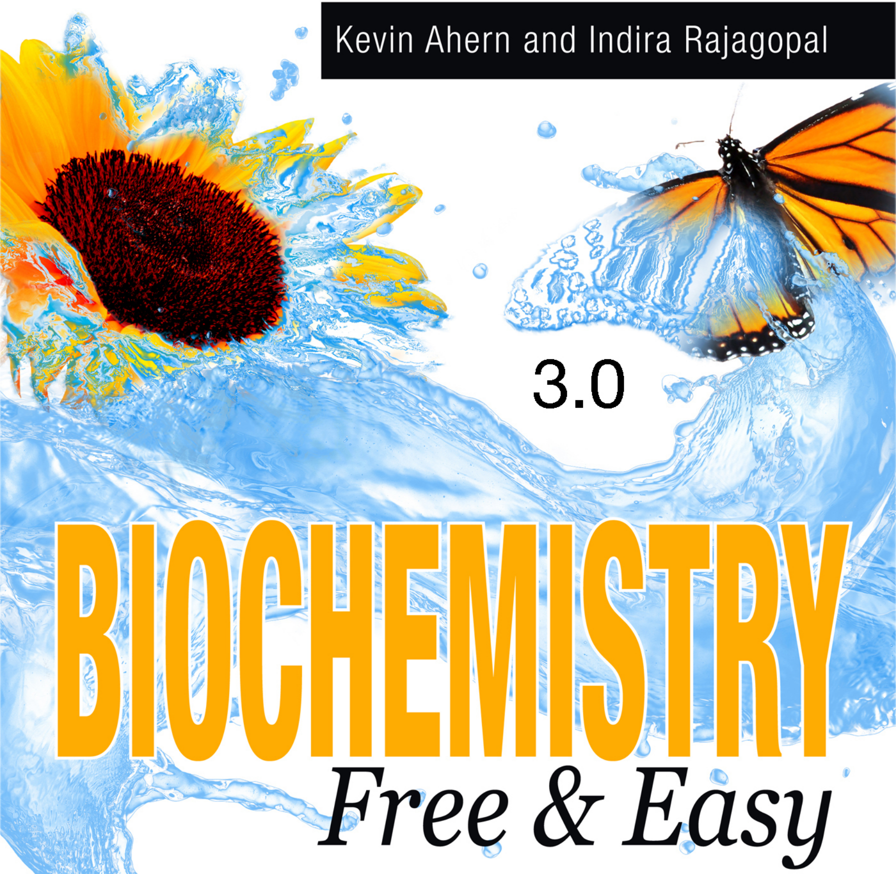
Kevin Ahern and Indira Rajagopal

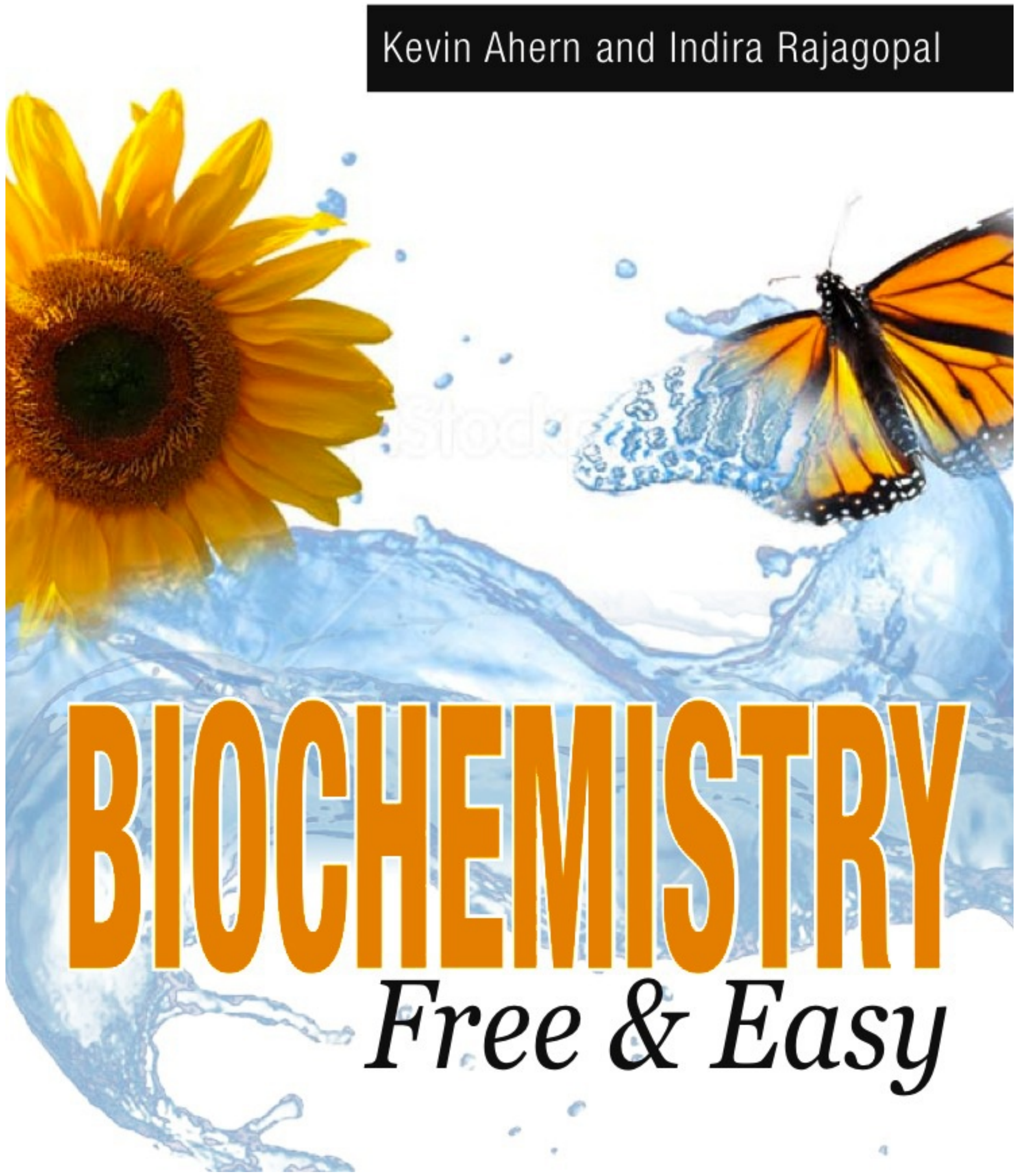


3.0

BIOCHEMISTRY

Free & Easy

Dedication & Credits



Biochemistry Free and Easy ©2012, 2013
Kevin Ahern & Indira Rajagopal / All rights reserved
Version 2.0

Biochemistry Free and Easy is dedicated to all of the people who have inspired us. Kevin would like to acknowledge his mentors, Dr. E.C. Nelson from Oklahoma State University and Dr. George Pearson from Oregon State University. Both were instrumental in Kevin's career development and are both mentors and lifelong friends. Indira would like to thank her parents, who passed on to her their love for the life sciences and nerdy jokes, through both their DNA and their example.

Dr. E.C. Nelson

On the pages that follow are photographs of students, past and present, who have inspired us and continue to inspire us to teach biochemistry. Not pictured, but included in this list are Beth Dunfield, Dr. Brian Orth, Dr. Jill Mooney, Dr. Jeremy Gregory, Dr. Keith Hazleton, Marsha Lampi, Dr. Tasha Ludwick, Thomas Pitts, Varunika Bhargava, and Zebulon Jones. The students here are a small subset of the thousands of students who have touched our lives and that we are grateful to have had the chance to work with at OSU.

Dr. George Pearson

Please note that though the photos are small, users can enlarge them by touching them on an iPad.

All images credited to Wikipedia have license information on the Copyright section beginning HERE.

Alex Aljets

Allex Hadduck

Amanda Schwartz

Amber Bannon

Dr. Amber Leis

Amini Thakor

Ana Brar

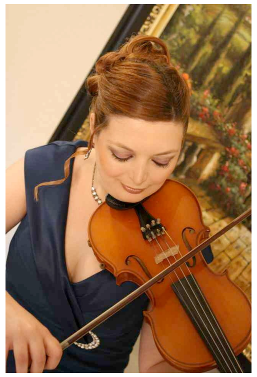
Dr. Anahita Fallahi

Andres Cardenas

Angel Garbarino

Dr. Anna Hsu

Anna Vigeland


Dr. Anne-Marie Corgan

Dr. April von Allmen

Ashley Meganck

Ashley Swander

Bailey Lindenmaier

Bonnie Buckingham

Dr. Bory Kea

Brett Bemer

Caitlin Crimp

Callia Palioca

Carly Dougher

Casey Luce & Elyssa Ackerman

Cassondra Pittman

Chelsea Parker

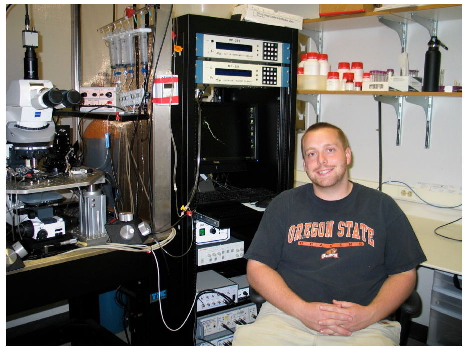
Chitra Patel

Chris Brown

Dr. Chrissy Lamun

Chrissy Murphy

Dr. Crystal Hammer



Daniel Zollinger

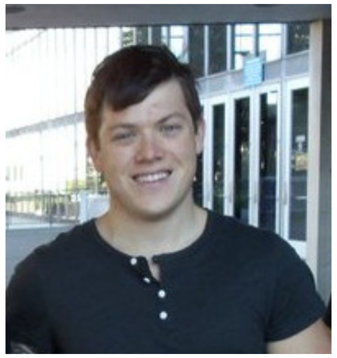
Danya Rumore

David Stanley

Dawn Gaudenti

Deepthi Ennamuri

Denise Knaebel



Derek Scott

Dr. Derek Youngblood

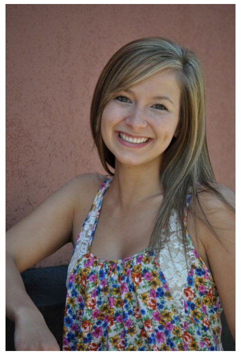
Desiree Boltz

Drew Calhoun

Duy Pham

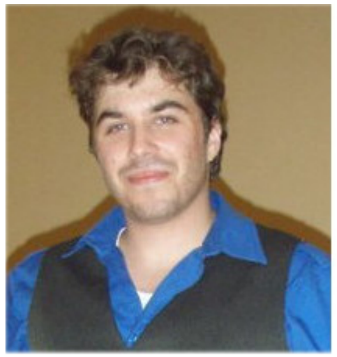
Emilie Servin

Eric Brooks



Erika Snow

Dr. Gautam Mankaney



Georgi Mitev

Hank McNett

Dr. Heather Bolstad

Heather Hodnett

Helen Hobbs

Ishan Patel

Dr. Jenna Donaldson

Jenna Smith

Jennifer Coppersmith

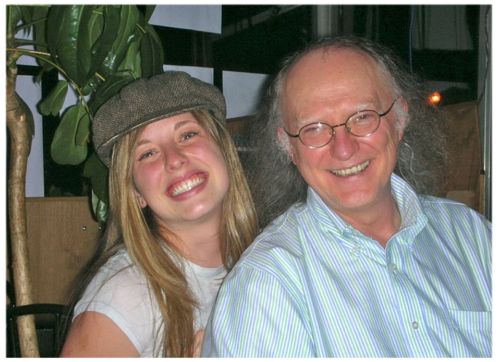
Dr. Jennifer Shepard

Jenny Moser Jurling

Jenny Tran, Andrea Virvara and Valeria Ursu

Jessica Goman

Jessica Kristoff

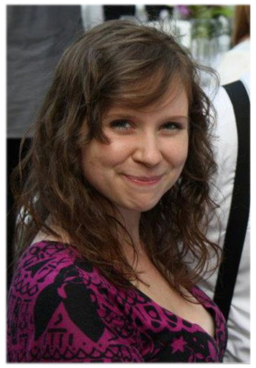

Jessica Page with Kevin Ahern

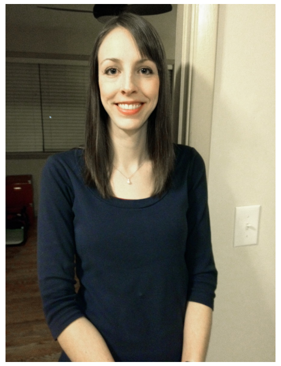
Jessica Taylor

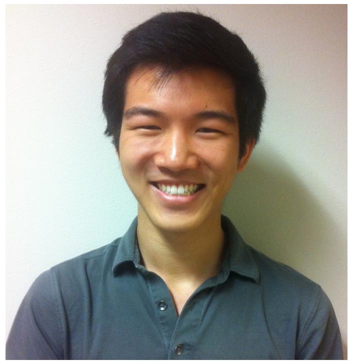
Jessica Thorpe

John Davidson

Josha Woodward

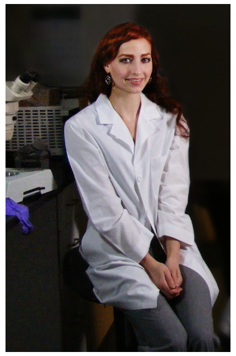

Julia Armendariz


Julia Sobolik Montes

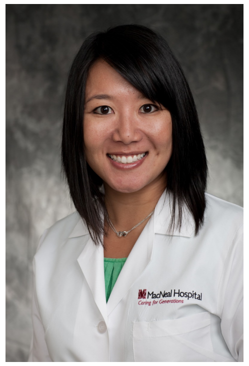

Justin Zhang

Kaalindi Misra

Kara Cardwell


Kate Bateman

Kate Posposil


Dr. Kathy Tran Shaw

Katie Brempelis

Katie Lebold

Kelsey Drewry

Dr. Kevan Stanton

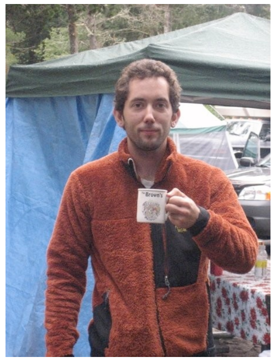
Dr. Kyle Shaver

Dr. Laura Miller

Leanna Mah

Lisa Weller

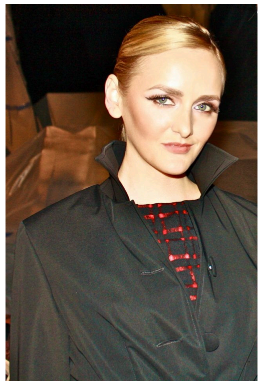
Loni Mandigo



Loren Brown

Lotti Alvord

Mallori Jirikovic



Dr. Mandi Kremer

Dr. Marie Strand

Mark Hall

Matt Lewis and Christine Schneider

Megan Cook

Meghan Krueger

Melodie Machovina

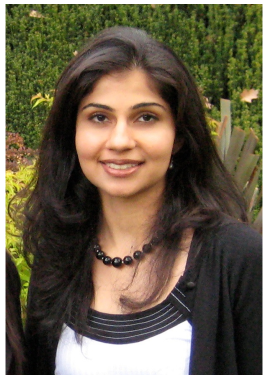
Michelle Buchanan

Michelle Nguyen

Dr. Michinao Hashimoto

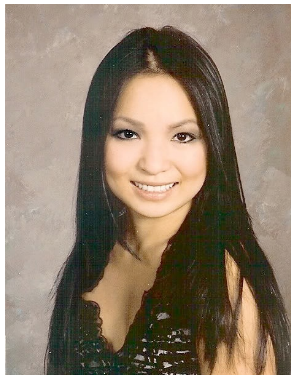
Dr. Minh Ho

Minhazur Sarker



Dr. Monika Arora Sanghavi

Dr. Nancy Jade Lee



Ngan Nguyen

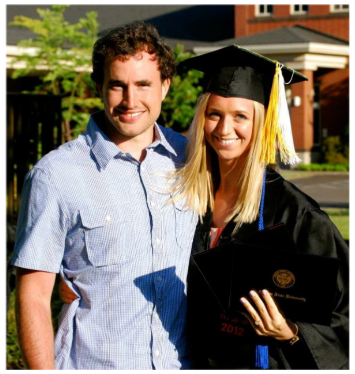
Nick Lowery

Nicolette O’Donnell

Dr. Nima Motamedi

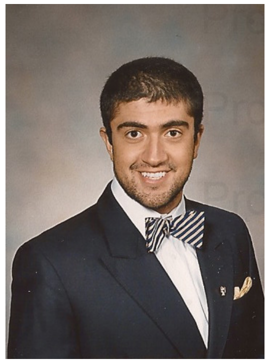
Omar Rachdi

Oresta Tolmach

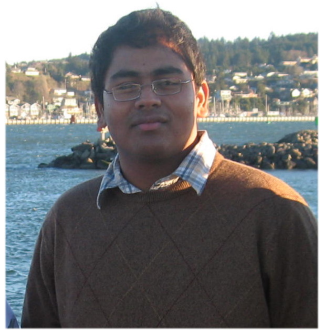


Paul Jones and Hannah Raines

Rachel Aazzerah



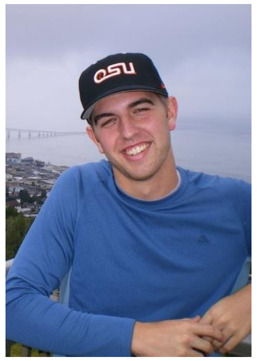
Rakan Khaki



Raviteja Madhira

Robbie Lamb

Robyn Ikehara Murakawa



Ryan Derrah

Sam Schuberg

Dr. Sara Haidar

Sarah and Michael Layoun

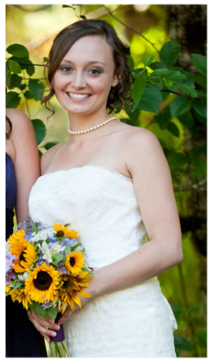
Sarah Ferrer & Emily Pickering

Sarah Lindsley

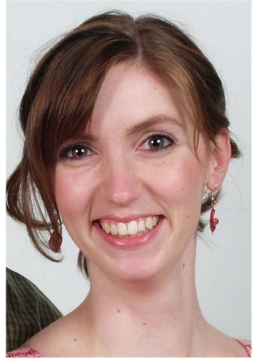
Dr. Sathya Sriram

Shannon Goff

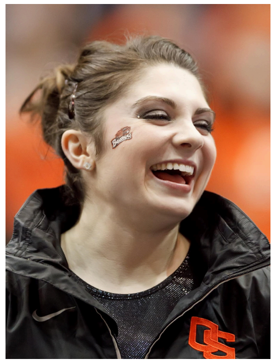
Dr. Shawn Werner



Shayna Rogers



Stephanie Duckett



Stephanie McGregor

Steve Davee

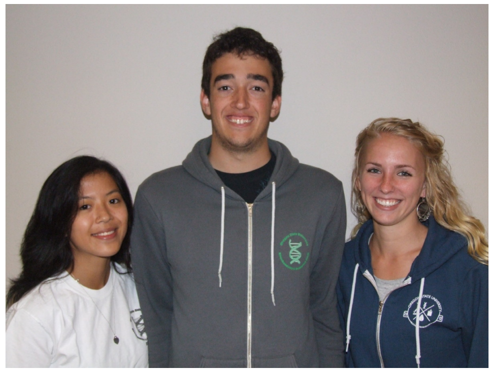
Steven Craig Brantley

Sydney Radding

Dr. Tara Humphrey

Tari Tan

Theresa & Maria Nguyen


Thi Nguyen, Justin Biel & Margo Roemeling

TJ Brodeur

Dr. Tom Sharpton

Tony Rianprakaisang

Tristan Wagner

Dr. Valerie Bomben

Valerie Mullen

Vihangi Hindagolla

Wren Patton

Yesenia Correa

Zoe Gombart

Credits

We are indebted to several people for their assistance in putting together this book.

Jana Rade is a generous, wonderfully talented artist who created the beautiful cover in the spirit of this book - for FREE, which was great because we didn't have much of a budget. Her graphic design business is **impact studios** and she can be reached at jrade@impactstudiosonline.com. Please support Jana's Web site at www.impactstudiosonline.com

Martha Jane Baker, an extraordinarily talented young Oregon State University student who created, or oversaw the creation of, all of the original artwork here. Please support her Web site at the following URL - www.mjbakerartist.com. She was assisted by Alyssa Hughes. Student wages for Martha and Alyssa were partly provided by a faculty grant to Kevin from the L.L. Stewart fund supporting faculty efforts that improve teaching at OSU.

David Simmons

David Simmons, **Liz Bacon**, **Barbara & Neal Gladstone**, and **Tim Karplus** brought the **Metabolic Melodies** to life with their delightful recordings that neither of us could ever dream of doing. David is currently a medical student at Oregon Health and Science University and will soon be Dr. David Simmons. Barbara and Neal Gladstone are wonderful friends of ours who can be reached through their musical Web site - www.nealgladstone.com

Liz Bacon

Indira and Kevin are faculty members in the Department of Biochemistry and Biophysics (link HERE) at Oregon State University (link HERE) where we teach biochemistry, molecular biology and Honors biology to hundreds of terrific students each year and write verses and songs about biochemistry called **Metabolic Melodies**, many of which were used here.

We decided when we created this book that information should be as freely available as possible. We appreciate your support (financial or otherwise) to say thanks for our efforts. Ways to do this include buying a calendar (HERE) or the "***The Splendidest Ever Metabolic Melodies***" songbook (HERE), registering for our e-campus classes at Oregon State University (HERE, HERE, HERE, and HERE), and/or sending us a message telling us how you've used the book. You can also join and **LIKE** our Facebook page for ***Biochemistry Free and Easy*** HERE.

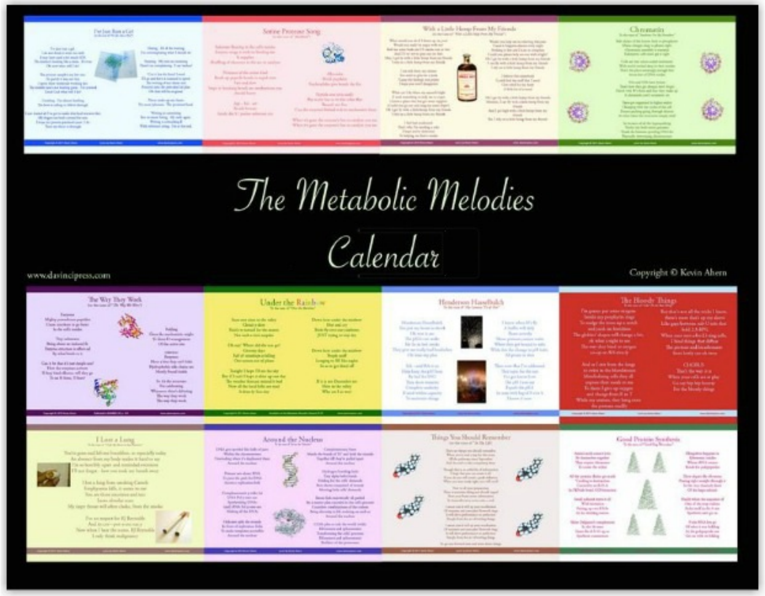
Our email addresses are

kevin@davincipress.com

indira@davincipress.com

You can view our biochemistry courses as follows below:

BB 350 - HERE / Syllabus HERE
BB 450/550 - HERE / Syllabus HERE
BB 451/551 - HERE / Syllabus HERE
BB 100 - HERE / Syllabus HERE

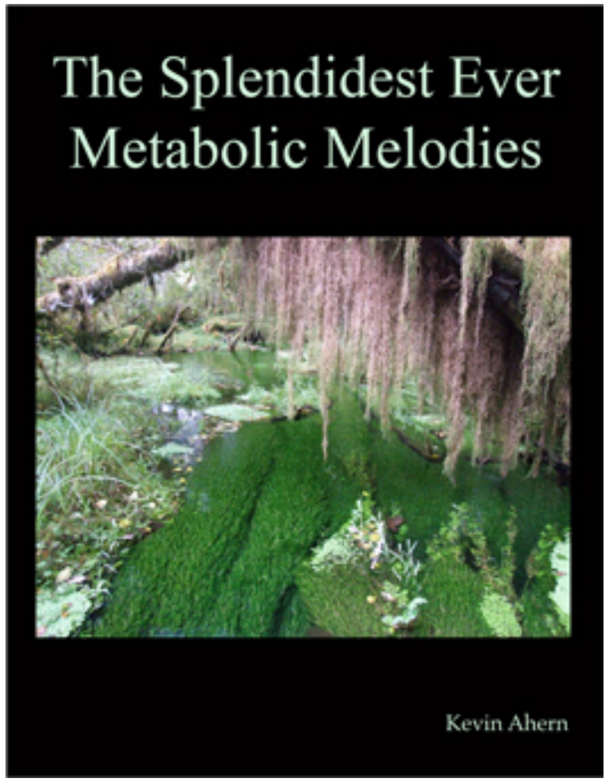


We have a Web site full of **Metabolic Melodies** and **Limericks** that you may enjoy. In the spirit of this book, everything there is free too. The URL is

http://www.davincipress.com/metabmelodies.html

Kevin has also released a book of limericks titled "**A Limerick A Day For A Year**" More information about that HERE.

He also a few YouTube music videos he is proud of. They include an anti-smoking video called "***I Lost a Lung (from smoking Camels)***" and is to the tune of "*I Left My Heart in San Francisco*" The link is HERE. The Oregon rain gets noted in "***Let It Rain***" (to the tune of "*Let It Snow*") HERE and "***Around the Nucleus***" (to the tune of "*Across the Universe*") is HERE. Last, Kevin has a series of video workshops of interest to pre-medical students. These can be accessed HERE



Chapter 1

Cells, Water, and Buffers

Welcome to our Biochemistry text. We're glad you're here. In this chapter we introduce the subject and talk about the scientific aspects of the most important and most abundant liquid on the face of the Earth - water.

Cells, Water, and Buffers

Introduction

Cells: The Bio of Biochemistry

Water, Water Everywhere

Buffers Keep the Cellular Environment Stable

Henderson-Hasselbalch

Introduction

Welcome to Biochemistry Free and Easy. As biochemistry instructors, we are always delighted when anyone shows an interest in our favorite subject. Helping students understand and enjoy biochemistry is our motivation for writing this book. It is not our intention to provide a comprehensive account of the chemical basis of life. Instead, we have tried to help students understand this fascinating subject by focusing on some key topics and concepts. This pared-down approach can be helpful for novices who might otherwise lose sight of important organizing principles in a sea of detail. The electronic format has also allowed us to provide multimedia links, in the form of video lectures and biochemistry songs to help students learn the subject. Best of all, this format makes it practical for us to distribute the book at no cost to anyone who wishes to learn basic biochemistry.

Biochemistry is a relatively young science, but the rate of its expansion has been truly impressive. This rapid pace of discoveries, which shows no signs of slowing, is reflected in the steady increase in the size of biochemistry text books, most of which top a thousand pages and undergo revisions every couple of years to incorporate new

On these pages our aim's to extract
Knowledge out of a mountain of facts
Missing excessive data
Will not really matta
If our students can learn and relax

findings. These full-scale texts offer an enormous amount of information and serve as invaluable resources. Those who need the greater level of detail and broader coverage that these books provide have many choices available in any good bookstore.

As certified (some might say, certifiable) biochemistry nerds and unrepentant lovers of corny jokes, we firmly believe that students can have fun while learning the subject. Toward this end, we have sprinkled each chapter with rhymes and songs that we hope will have you learning biochemistry happily. The format of the book as available for the iPad, allows readers to click on figures to enlarge them, watch video lectures relevant to each topic, listen to the songs in the book, like the one above, and link out to the internet to find more information

Biochemistry, Biochemistry

To the tune of "*Oh Christmas Tree*"

Biochemistry Biochemistry
I wish that I were wiser
I feel I'm in way o'er my head
I need a new advisor

My courses really shouldn't be
Such metabolic misery
Biochemistry Biochemistry
I wish that I were wiser

Biochemistry Biochemistry
Reactions make me shiver
They're in my heart and in my lungs
They're even in my liver

I promise I would not complain
If I could store them in my brain
Biochemistry Biochemistry
I wish that I were wiser

Biochemistry Biochemistry
I'm truly in a panic
Your mechanisms murder me
I should have learned organic

For all I have to memorize
I ought to win the Nobel Prize.
Biochemistry Biochemistry
I wish that I were wiser

Biochemistry, Biochemistry

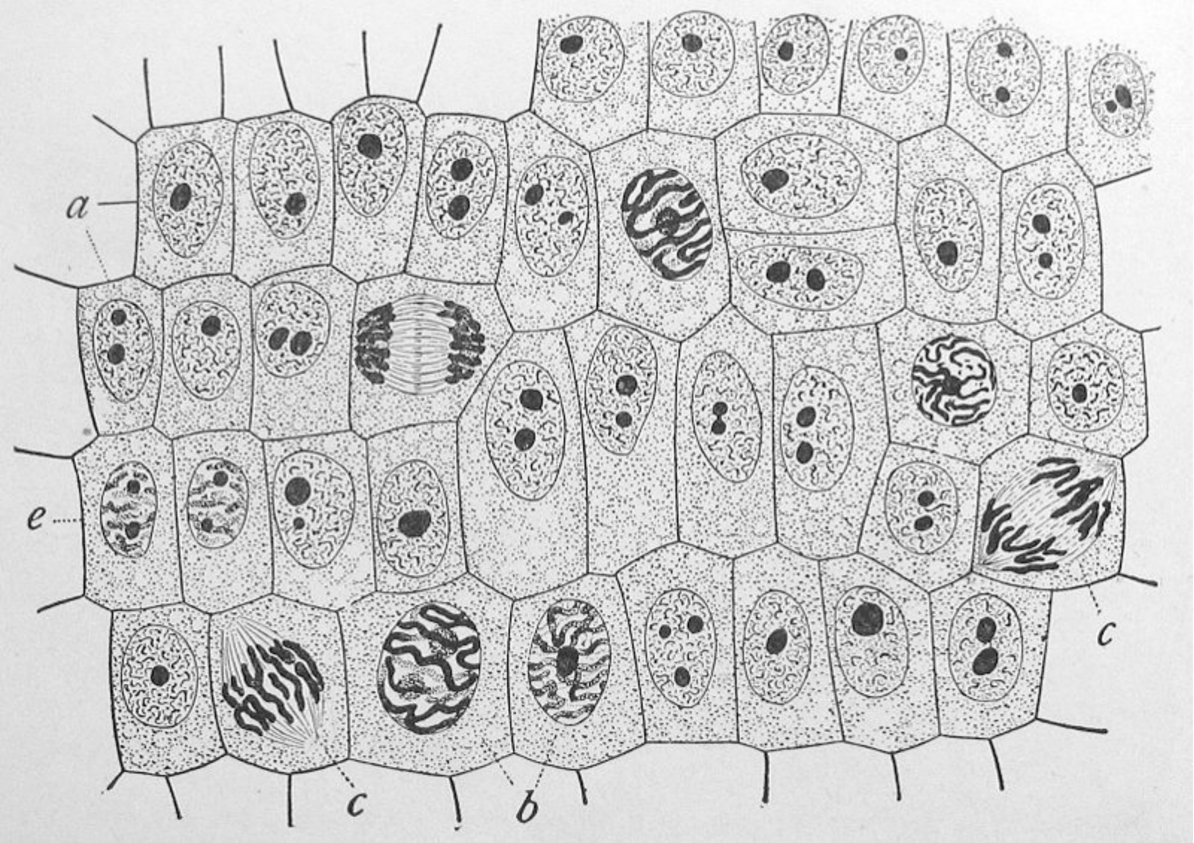
Recorded by Tim Karplus.
Lyrics by Kevin Ahern

simply by clicking on any term. If you are using a PDF version of this book, you will still be able to use the links to the video lectures. Also, though you cannot listen to the songs by clicking on them in the PDF version, you can download them HERE. We hope you find these features useful and that they help you learn biochemistry.

Cells: The Bio of Biochemistry

Biochemistry happens inside organisms and possibly, the most obvious thing about living organisms is their astounding diversity. If living things are so varied, it seems reasonable to ask whether their chemistry is, too.

The invention of the microscope opened up a whole new world of microscopic organisms while also providing the first clue that living organisms had something in common- all living things are made up of cells. Some cells are “lone rangers” in the form of unicellular entities, such as bacteria and some protists. Cells are also the building blocks of more complex organisms (like humans, wombats, and turnips).



Onion cells

from Wikipedia

As increasingly powerful microscopes became available, it was possible to discern that all cells fell into one of two types- those with a nucleus and other sub-cellular compartments like mitochondria and lysosomes, termed eukaryotes, and those that lack such internal compartmentation, the prokaryotes. Some eukaryotes, such as yeast, are unicellular, while others, including animals and plants are multicellular. The prokaryotes may be divided into two very broad categories, the bacteria and the archaeans.

Excuse me for feeling superia
To the life forms we call the bacteria
Students know very well
There are no organelles
To be found in their tiny interia

One can find living cells almost everywhere on earth - in thermal vents on the ocean floor, on the surface of your tongue and even in the frozen wastes of the Antarctic. Some cells may

Extremophiles

from Wikipedia

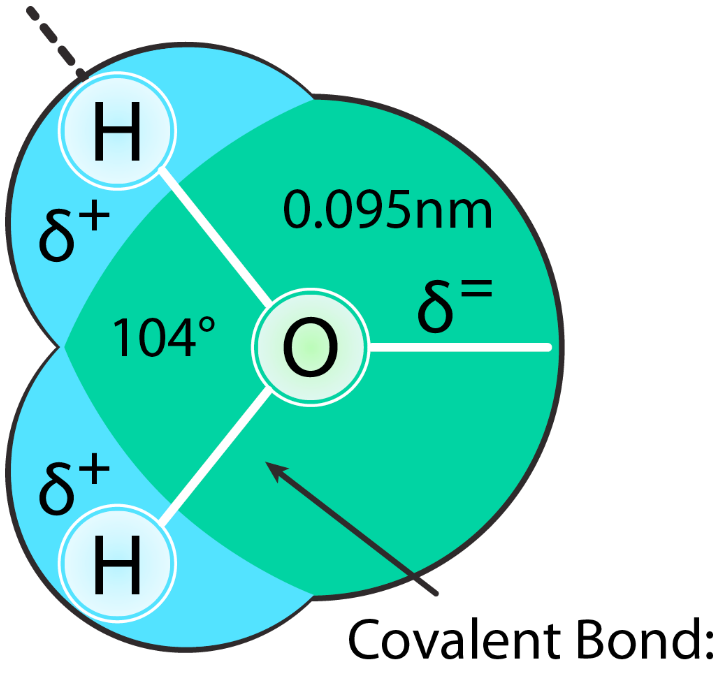
have even survived over two years on the moon[1]. Yet, despite their diversity of appearance, habitat, and genetic composition, cells are not as different from each other as you might expect. At the biochemical level, it turns out that all cells are more alike than they are different. A great simplifying feature of biochemistry is that many of the reactions are universal, occurring in all cells. For example, most bacteria process glucose in the same 10-step pathway that plant, animal, and fungal cells do. The genetic code that specifies the amino acids encoded by a nucleic acid sequence is interpreted almost identically by all living cells, as well. Thus, the biochemical spectrum of life is (mercifully) not nearly as broad or as complicated as the evolutionary spectrum. Where cells differ significantly in processes/reactions, we will note these differences.

Water, Water, Everywhere

Click HERE for Kevin's Introductory Lecture on YouTube

Vital for life, water is by far the most abundant component of every cell. To understand life, we must, therefore, understand the basics of water, because everything that happens in cells, even reactions buried deep inside enzymes, away from water, is influenced by water's chemistry.

from Wikipedia



We start with simple properties. The molecule has a sort of wide 'V' shape (the H-O-H angle is 104°) with uneven sharing of electrons between the oxygen and the hydrogens. The hydrogens, as a result, are described as having a partial positive charge and the oxygen has a partial negative charge. These tiny partial charges allow the formation of what are described as hydrogen bonds, which occur when the partial positive charge of one atom is attracted to the partial negative of another. In water, that means the hydrogen of one water molecule will be attracted to the oxygen of another. Hydrogen bonds play essential roles in proteins, DNA, and RNA, as well, as we shall see.

Buffers Keep the Cellular Environment Stable

Water can ionize to a slight extent (10^{-7} M - about 6 molecules per 100 million of pure water) to form H^+ (proton) and OH^- (hydroxide). We measure the proton concentration of a solution with pH, which we define as the negative log of the proton concentration.

$$pH = -Log[H^+]$$

If the proton concentration, $[H^+]= 10^{-7}$ M, then the pH is 7. We could just as easily measure the hydroxide concentration with the pOH by the parallel equation,

$$pOH = -Log[OH^-]$$

In pure water, dissociation of a proton from it creates a hydroxide, so the pOH of pure water is 7, as well. This also means that

$$pH + pOH = 14$$

Why do we care about pH? Because biological molecules can, in some cases, be exquisitely sensitive to changes in it. As the pH of a solution changes, the charges of molecules in the solution can change, as you will see. Changing charges on biological molecules, especially proteins, can drastically affect how they work and even whether they work at all.

Weak Acid pKa Values

Name	Chemical Structure of Acid	Chemical Structure of Salt	pKa
Acetic Acid	CH_3COOH	CH_3COO^-	4.76
Formic Acid	$HCOOH$	$HCOO^-$	3.75
Lactic Acid	$CH_3CHOHCOOH$	$CH_3CH—HCOO^-$	3.86
Pyruvic Acid	$CH_3COCOOH$	$CH_3C—COO^-$	2.50
Oxalic Acid (1)	$HOOC—COOH$	$HOOC—COO^-$	1.23
Oxalic Acid (2)	$HOOC—COO^-$	$OOC—COO^-$	4.19
Carbonic Acid (1)	H_2CO_3	HCO_3^-	6.37
Carbonic Acid (2)	HCO_3^-	CO_3^{2-}	10.20
Malic Acid (1)	$HOOC—CH_2—CHOH—COOH$	$HOOC—CH_2—CHOH—COO^-$	3.40
Malic Acid (2)	$HOOC—CH_2—CHOH—COO^-$	$^-OOC—CH_2—CHOH—COO^-$	5.26
Malonic Acid (1)	$HOOC—CH_2—COOH$	$HOOC—CH_2—COO^-$	2.83
Malonic Acid (2)	$HOOC—CH_2—COO^-$	$^-OOC—CH_2—COO^-$	5.69
Phosphoric Acid (1)	H_3PO_4	$H_2PO_4^-$	2.14
Phosphoric Acid (2)	$H_2PO_4^-$	HPO_4^{2-}	7.20
Phosphoric Acid (3)	HPO_4^{2-}	PO_4^{3-}	12.40
Succinic Acid (1)	$HOOC—CH_2—CH_2—COOH$	$HOOC—CH_2—CH_2—COO^-$	4.21
Succinic Acid (2)	$HOOC—CH_2—CH_2—COO^-$	$^-OOC—CH_2—CH_2—COO^-$	5.63

Now, because protons and hydroxides can combine to form water, a large amount of one will cause there to be a small amount of the other. Why is this the case? In simple terms, if I dump 0.1 moles of H^+ into a pure water solution, the high proton concentration will react with the relatively small amount of hydroxides to create water, thus reducing hydroxides. Similarly, if I dump excess hydroxide (as NaOH, for example) into pure water, the proton concentration falls for the same reason.

Chemists use the term "acid" to refer to a substance which has protons that can dissociate (come off) when dissolved in water. They use the term "base" to refer to a substance that can absorb protons when dissolved in water. Both acids and bases come in strong and weak forms. (Examples of weak acids are shown on the previous page.) Strong acids, such as HCl, dissociate completely in water. If we add 0.1 moles of HCl to a solution to make a liter, it will have 0.1 moles of H^+ and 0.1 moles of Cl^-. There will be no remaining HCl when this happens. A strong base like NaOH also dissociates completely into Na^+ and OH^-.

Weak acids and bases differ from their strong counterparts. When you put one mole of acetic acid (HAc) into pure water, only about 4 in 1000 HAc molecules dissociate into H^+ and Ac^-. Thus, if I start with 1000 HAc, I will end up with 996 HAc and 4 each of H^+ and Ac^-. Clearly, weak acids are very different from strong acids. Weak bases behave similarly, except that they accept protons, rather than donate them.

You may wonder why we care about weak acids. You may never have thought much of weak acids when you were in General Chemistry. Your instructor described them as buffers and you probably dutifully memorized the fact that "buffers are substances that resist change in pH" without really learning what it meant. We will not allow that to happen here.

Click HERE for Kevin's
Buffer Lecture
on YouTube

Weak acids are critical for life because their affinity for protons causes them to behave like a UPS. We're not referring to the UPS that is the United Parcel Service®, but instead, to the encased battery backup systems for computers called Uninterruptible Power Supplies that kick on to keep a computer running during a power failure. Your laptop battery is a UPS, for example. We can think of weak acids as Uninterruptible Proton Suppliers within certain pH ranges, providing (or absorbing) protons as needed. Weak acids thus help to keep the H^+ concentration (and thus the pH) of the solution they are in relatively constant.

On this chemical fact we must dwell
H-A-C's just not like H-C-L
It always negotiates
Before it dissociates
To bid every proton farewell

Consider the acetic acid (acetate) system. Here is what happens when HAc dissociates

$$HAc <=> H^+ + Ac^-$$

As noted, about 4 in 1000 HAc molecules come apart. However, what if one started adding hydroxyl ions (by adding a strong base like NaOH) to the solution with the HAc in it? As the added OH^- ions reacted with the H^+ ions to make water, the concentration of H^+ ions would go down and the pH would go up. However, in contrast to the situation with a solution of pure water, there is a backup source of H^+ available in the form of HAc. Here is where the UPS function kicks in. As protons are taken away by the added hydroxyl ions (making water), they are partly replaced by protons from the HAc. This is why a weak acid is a buffer. It resists changes in pH by releasing protons to compensate for those "used up" in reacting with the hydroxyl ions.

Henderson-Hasselbalch

It is useful to be able to predict the response of the HAc system to changes in H^+ concentration. The Henderson-Hasselbalch equation defines the relationship between pH and the ratio of Ac^- and HAc. It is as follows

$$pH = pK_a + \log ([Ac^-]/[HAc])$$

This simple equation defines the relationship between the pH of a solution

I confess I am pleased to possess
In my buffers a strong UPS
Giving H's when needed
Grabbing same when exceeded
So my cells don't get proton distressed

and the ratio of Ac^- and HAc in it. The new term, called the pK_a, is defined as

$$pK_a = -\text{Log } K_a\text{, just as } pH = -\text{Log } [H^+]$$

The K_a is the acid dissociation constant and is a measure of the strength of an acid. For a general acid, HA, which dissociates as

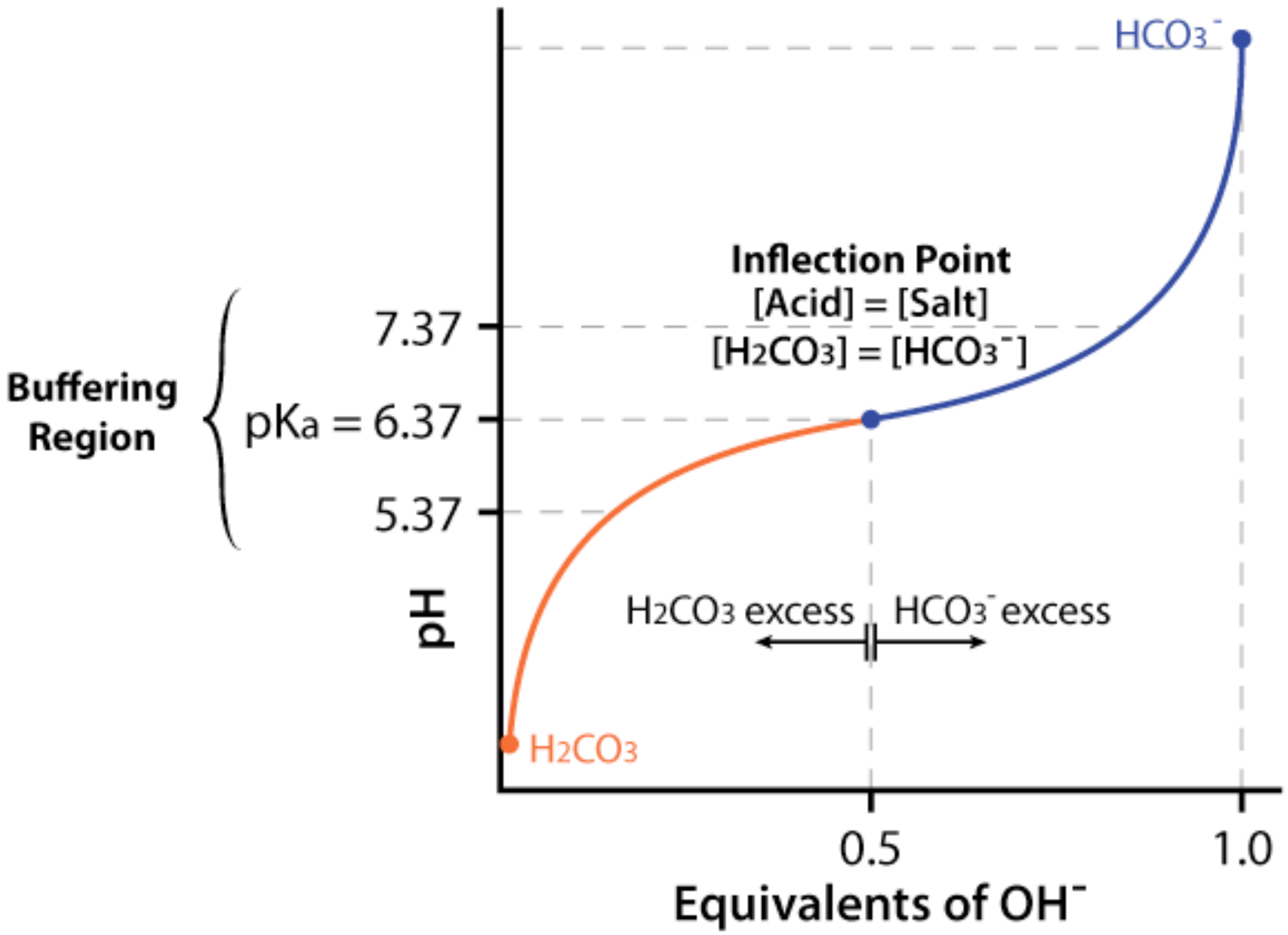
$$HA \Leftarrow\Rightarrow H^+ + A^-,$$

$$Ka = [H^+][A^-]/[HA]$$

Thus, the stronger the acid, the more protons that will dissociate from it and the larger the value its Ka will have. Large values of K_a translate to lower values of pK_a. As a result, the lower the pKa value is for a given acid, the stronger the acid is.

Please note that pK_a is a constant for a given acid. The pK_a for acetic acid is 4.76. By comparison, the pK_a for formic acid is 3.75. Formic acid is therefore a stronger acid than acetic acid. A stronger acid will have more protons dissociated at a given pH than a weaker acid.

Now, how does this translate into stabilizing pH? The previous figure shows a titration curve. In this curve, the titration begins with the conditions at the lower left (very low pH). At a this pH, the HAc form predominates, but as more and more OH^- is added (moving to the right), the pH goes up, the amount of Ac^- goes up and (correspondingly), the amount of HAc goes down. Notice that the curve “flattens” near the pKa (4.76).



An example buffer system - carbonic acid/bicarbonate. Note that near pH 6.73, addition of OH^- results in only small pH changes. This is what a buffer does - resists changes in pH over certain ranges.

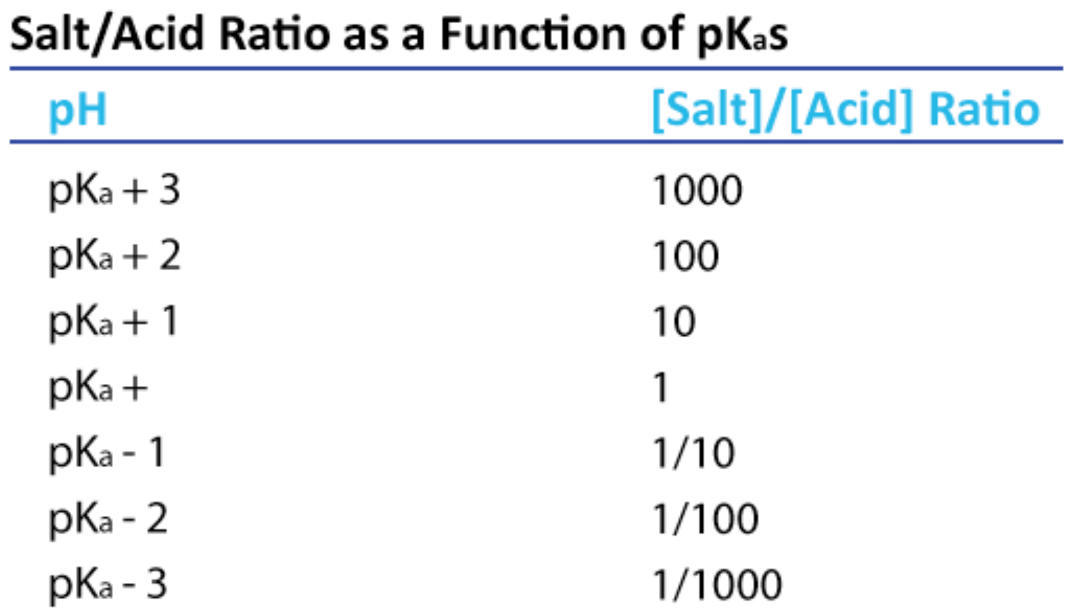
What this tells us is that the pH is not changing much (not going up as fast) as it did earlier when the same amount of hydroxide was added. The system is resisting a change in pH (not stopping

Why do we care about buffers? Buffers help to keep the pH of a solution from changing much, even when protons are added to it or removed from it. When you exercise, your muscles produce protons, which get dumped into the blood. If a buffer were not present, the pH of the blood would change drastically and you would likely die, since the acidification of your blood would denature/inactivate most of your enzymes

the change, but slowing it) in the region of about one pH unit above and one pH unit below the pKa. Thus, the buffering region of the acetic acid/acetate buffer is from about 3.76 to 5.76. It is maximally strong at a pH of 4.76.

Now it starts to become apparent how the buffer works. HA can donate protons when extras are needed (such as when OH$^-$ is added to the solution. Similarly, A$^-$ can accept protons when extra H$^+$ are added to the solution (adding HCl, for example). The maximum ability to donate or accept protons comes when

$$[A^-] = [HA]$$



Salt/Acid Ratio as a Function of pK$_a$s

pH	[Salt]/[Acid] Ratio
pK$_a$ + 3	1000
pK$_a$ + 2	100
pK$_a$ + 1	10
pK$_a$ +	1
pK$_a$ - 1	1/10
pK$_a$ - 2	1/100
pK$_a$ - 3	1/1000

To understand how well a buffer protects against changes in pH, consider the effect of adding .01 moles of HCl to 1.0 liter of pure water (no volume change) at pH 7, compared to adding it to 1.0 liter of a 1M acetate buffer at pH 4.76. Since HCl completely dissociates, in 0.01M (10^{-2} M) HCl you will have 0.01M H$^+$. For the pure water, the pH drops from 7.0 down to 2.0 (pH = -log(0.01M)).

By contrast, the acetate buffer's pH is 4.74. Thus, the pure water solution sees its pH fall from 7 to 2 (5 pH units), whereas the buffered solution saw its pH drop from 4.76 to 4.74 (0.02 pH units). Clearly, the buffer minimizes the impact of the added protons compared to the pure water.

It is important to note that buffers have capacities limited by their concentration. Let's imagine that in the previous paragraph, we had added the 0.01 moles HCl to an acetate buffer that had a

Why does the pKa give the pH of the maximum buffering capacity? From the Henderson-Hasselbalch equation, when pH = pKa, the log term (log{[A-]/[HA]) must be zero. For the log term to be zero, [A-] must equal [HA].

Henderson Hasselbalch

To the tune of "*My Country 'Tis of Thee*"

Henderson Hasselbalch
You put my brain in shock
Oh woe is me
The pKa's can make
Me lie in bed awake
They give me really bad headaches
Oh hear my plea

Salt - acid RA-ti-os
Help keep the pH froze
By buf-fer-ING
They show tenacity
Complete audacity
If used within capacity
To maintain things

I know when H's fly
A buffer will defy
Them actively
Those protons cannot waltz
When they get bound to salts
With this the change in pH halts
All praise to thee

Thus now that I've addressed
This topic for the test
I've got know-how
The pH I can say
Equals the pKa
In sum with log of S o'er A
I know it now

Recorded by David Simmons
Lyrics by Kevin Ahern and Indira Rajagopal

concentration of 0.01M and equal amounts of Ac^- and HAc. When we try to do the math in parallel to the previous calculation, we see that there are 0.01M protons, but only 0.005M A^- to absorb them. We could imagine that 0.005M of the protons would be absorbed, but that would still leave 0.005M of protons unbuffered. Thus, the pH of this solution would be approximately

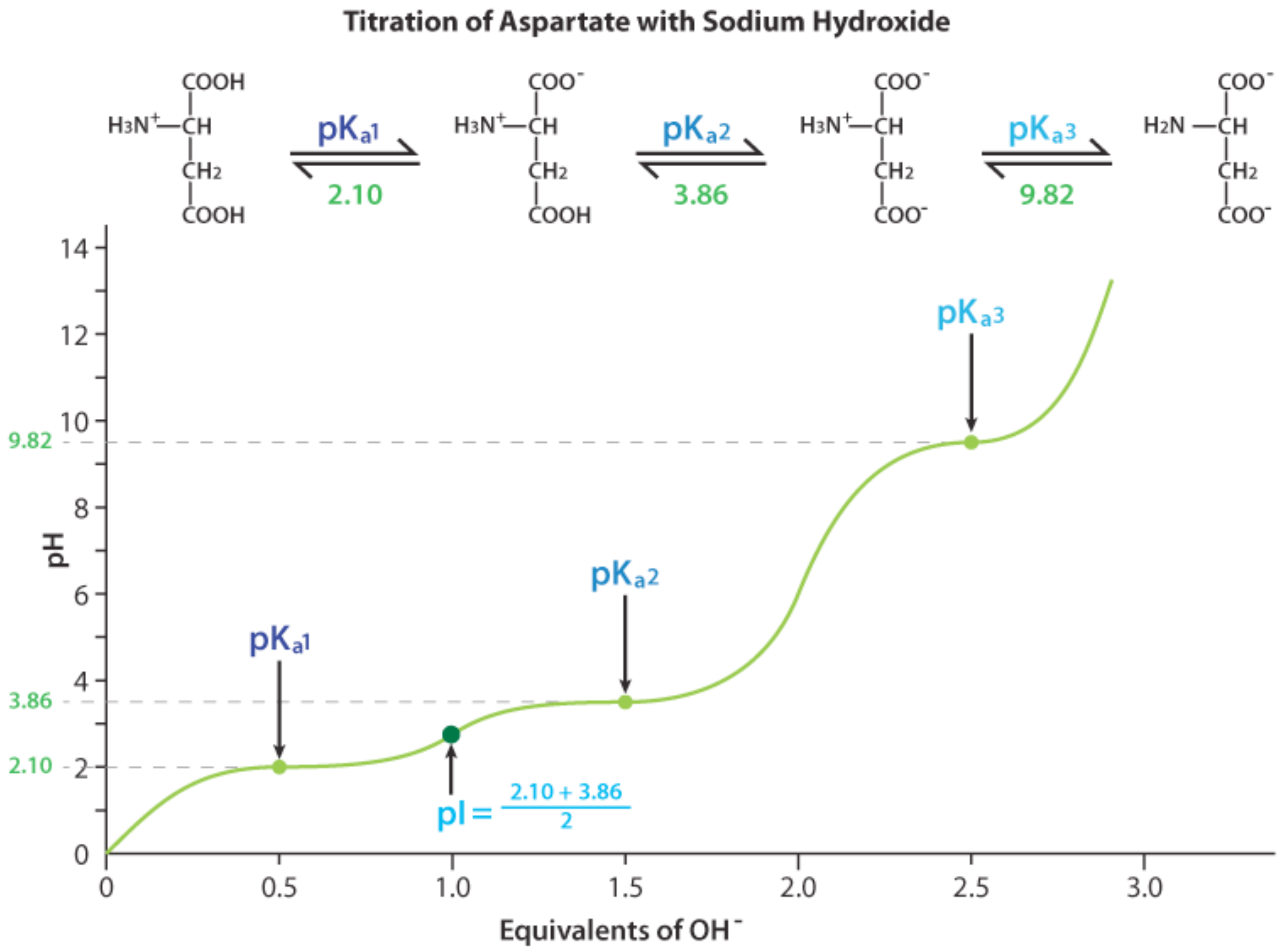
$$pH = -\log(0.005M) = 2.30$$

Exceeding buffering capacity dropped the pH significantly compared to adding the same amount of protons to a 1M acetate buffer. Consequently, when considering buffers, it is important to recognize that their concentration sets their limits. Another limit is the pH range in which one hopes to control proton concentration.

Now, what happens if a molecule has two (or more) ionizable groups? It turns out, not surprisingly, that each group will have its own pKa and, as a consequence, will tend to ionize at different pH values. The figure above right shows the titration curve for a simple amino acid, alanine. Note that instead of a single flattening of the curve, as was seen for acetic acid, alanine displays two such regions. These are individual buffering regions, each centered on the respective pKa values for the carboxyl group and the amino group.

If we think about alanine, it can have three possible charges: +1 (alpha carboxyl group and alpha amino group each has a proton), 0 (alpha carboxyl group missing a proton and alpha amino group has a proton) and -1 (alpha carboxyl group and alpha amino group each lacking a proton).

It surely cannot get much tougher
Than exceeding the range of your buffer
All those excess H-plusses
Will raise a big ruckus
As the falling pH makes you suffer



How does one predict the charge at a given pH for an amino acid? A good rule of thumb for estimating charge is that if the pH is more than one unit below the pKa for a group (carboxyl or amino), the proton is on. If the pH is more than one unit above the pKa for the group, the proton is off. If the pH is NOT more than one or less than one pH unit from the pKa, this simple assumption will not work. Further, it is important to recognize that these rules of thumb are estimates only. The pI (pH at which the charge of a molecule is zero) is an exact value calculated as the average of the two pKa values on either side of the zero region. It is calculated at the average of the two pKa values around the point where the charge of the molecule is zero.

Reference

1. http://www.lpi.usra.edu/lunar/missions/apollo/apollo_12/experiments/surveyor/

Around the Nucleus

To the tune of "*Across the Universe*"

DNA gets spooled like balls of yarn
Within the chromosomes
Unwinding when it's duplicated there
Around the nucleus

Primase sets down RNA
To pave the path for DNA
Across a replication fork

Complementarit-y rules (ahhhhhh)
DNA Pol-y-mer-ase
Synthesizing DNAs
RNA Pol-y-mer-ase
Making all the RNAs

Helicases split the strands
In front of replication forks
To make templates accessible
Around the nucleus

Complementary bases
Match the bonds of 'H' and hold the strands
Together till they're pulled apart
Around the nucleus
Hydrogen bonding fuels (ahhhhhhhhh)
Tiny alpha helix bands
Folding for the cells' demands
Beta sheets comprised of strands
Meeting all the cells' demands

Exons link majestically all guided
By a master plan encoded in the cell's genome
That's buried deep inside of me
Countless combinations of the codons
Bring diversity to life evolving on and on
Around the nucleus

Complexes rule the world (ahhhhhhhh)
Ribosomes and spliceosomes
Transforming the cells' genomes
Ribosomes and spliceosomes
Builders of the proteomes

YouTube video HERE

Recorded by David Simmons
Lyrics by Kevin Ahern

Jump to Chapter

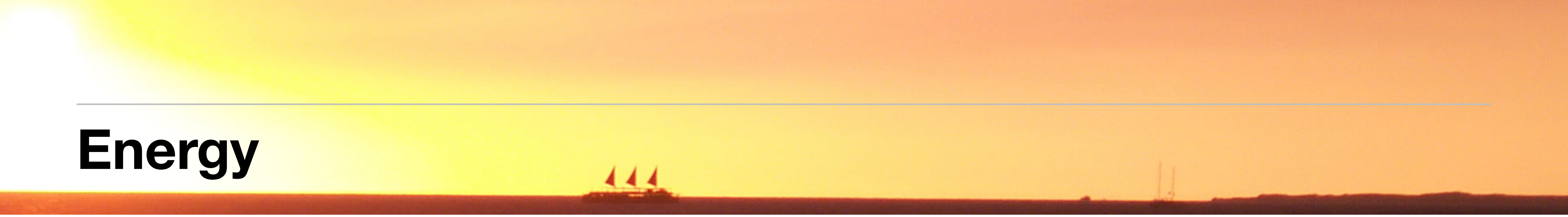
1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Chapter 2

Energy

In this chapter we discuss the most important factor necessary for life - energy.

RIP CURL
RIP CURL
★STAR★ BEACH BOYS
STAR BEACHBOYS
Surfing Lessons
Canoe Rides
Rentals:



Energy

- Introduction
- Oxidative Energy
- Oxidation vs. Reduction
- Energy Coupling
- Entropy and Energy
- Gibbs Free Energy
- Cellular Phosphorylations
    - Substrate Level Phosphorylation
    - Oxidative Phosphorylation
    - ATP Synthase
    - Photophosphorylation
    - Chloroplasts vs. Mitochondria
- Energy Efficiency
- Metabolic Controls
- Molecular Battery Backups

Introduction

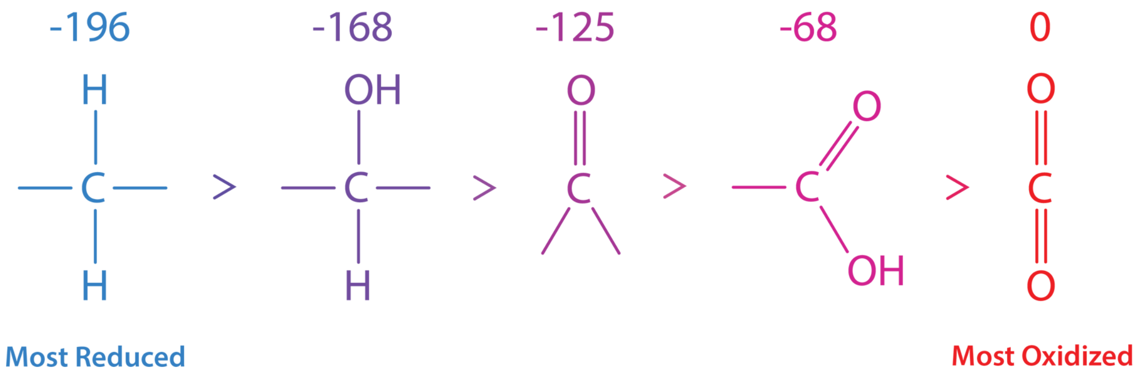
Living organisms are made up of cells, and cells contain many biochemical components such as proteins, lipids, and carbohydrates. But, living cells are not random collections of these molecules. They are extraordinarily organized or "ordered". By contrast, in the nonliving world, there is a universal tendency to increasing disorder. Maintaining and creating order in cells takes the input of energy.

Without energy, life is not possible. It is therefore important that we consider energy first in our attempt to understand biochemistry. Where does energy come from? Photosynthetic organisms can capture energy from the sun, converting it to chemical forms usable by cells. Heterotrophic organisms like ourselves get our energy from the food we eat. How do we extract the energy from the food we eat?

Oxidative Energy

The primary mechanism used by non-photosynthetic organisms to obtain energy is oxidation and carbon is the most commonly oxidized energy source. The energy released during the oxidative steps is “captured” in ATP and can be used later for energy coupling (see below). The more reduced a carbon atom is, the more energy can be realized from its oxidation. Conversely, the more oxidized a carbon atom is, the more energy it takes to reduce it.

Free energies of oxidation in KJ/mol


-196
-168
-125
-68
0
Most Reduced
Most Oxidized

In this series, the most reduced form of carbon is on the left. The energy of oxidation of each form is shown above it. Fatty acids are more reduced overall than sugars. This can also be seen by their formulas.

Palmitic acid = $C_{16}H_{34}O_2$

Glucose = $C_6H_{12}O_6$

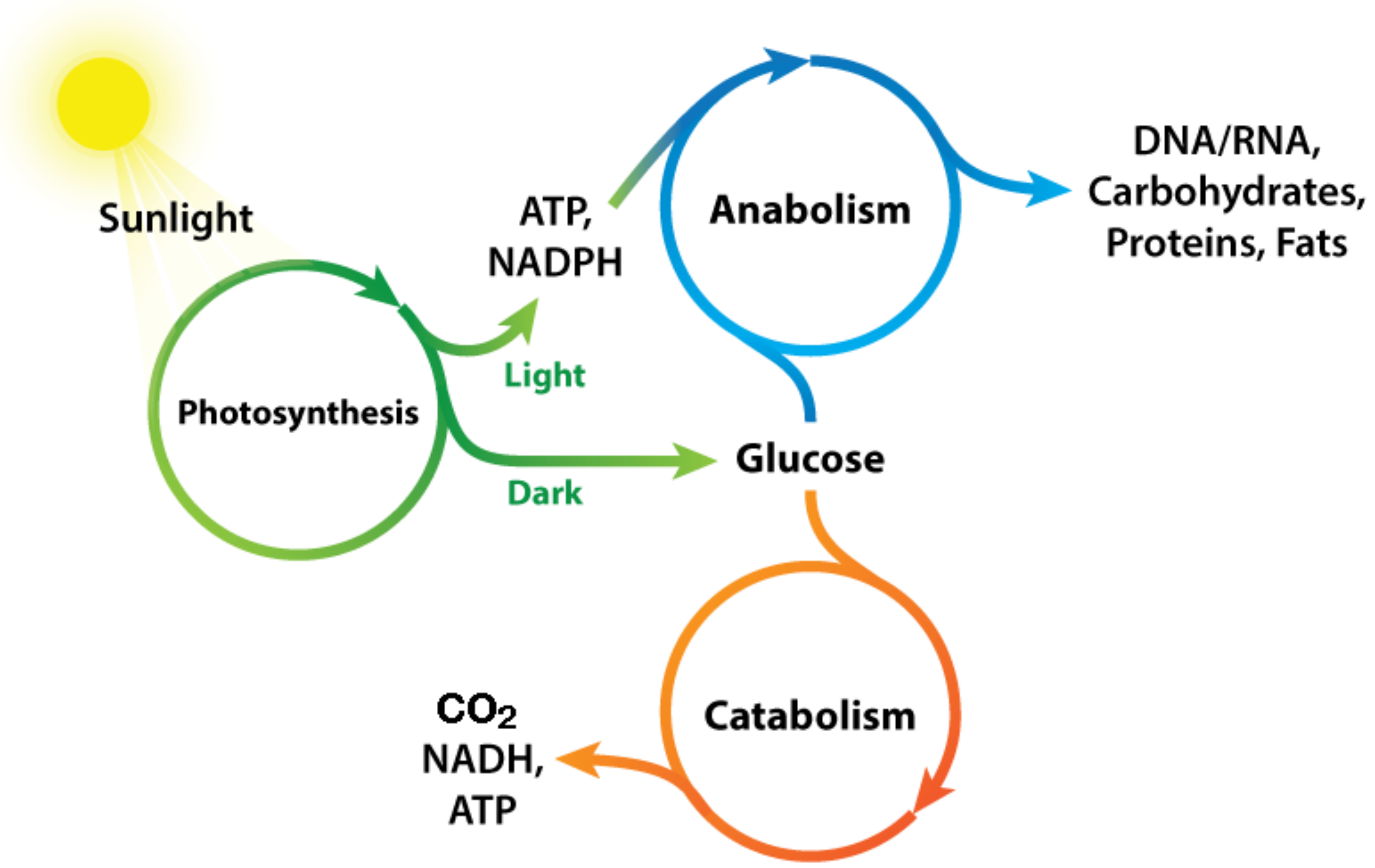
Palmitic acid only contains two oxygens per sixteen carbons, whereas glucose has six oxygen atoms per six carbons.

Consequently, when palmitic acid is fully oxidized, it generates more ATP per carbon (128/16) than glucose (38/6). It is because of this that we use fat as our primary energy storage material.

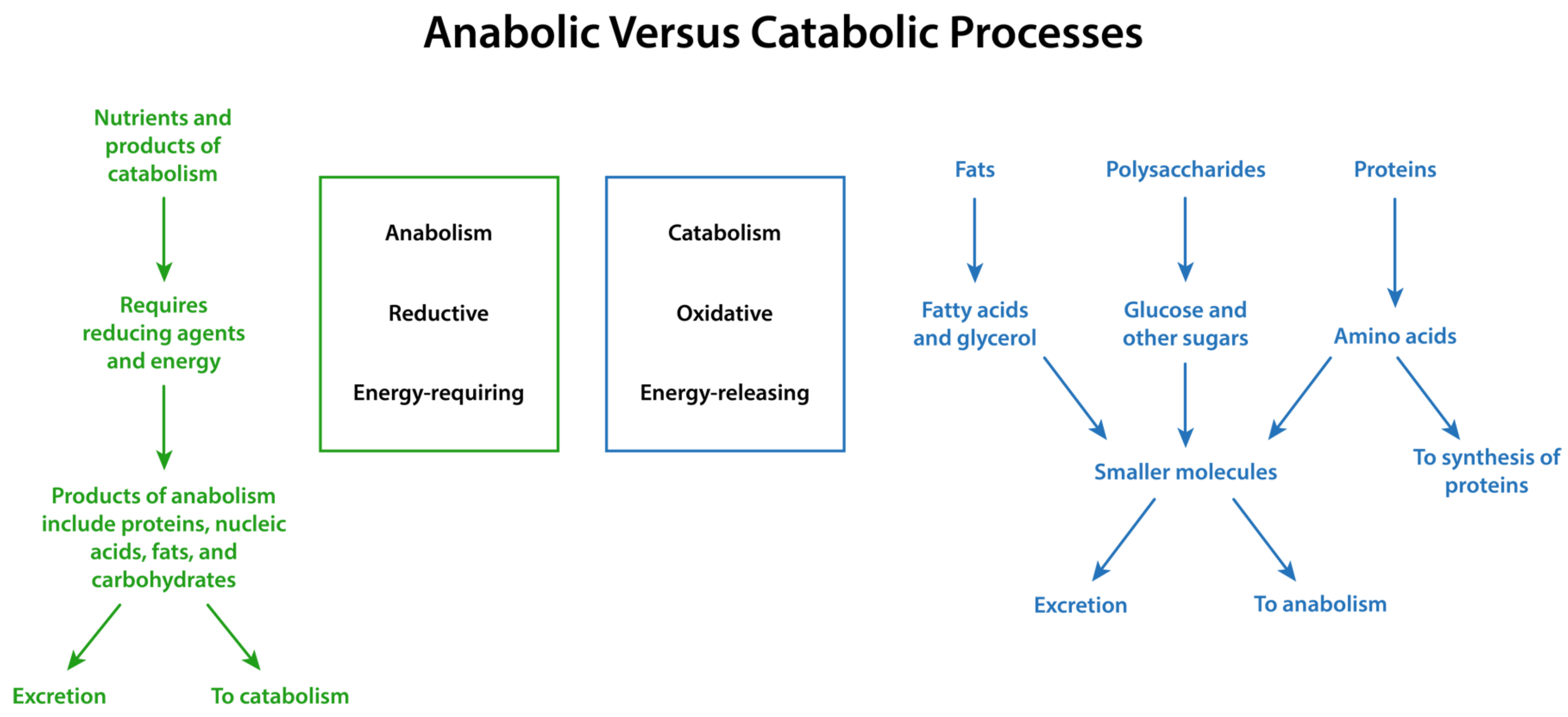
Oxidation vs. Reduction in Metabolism

Click HERE and HERE for Kevin's Metabolic Strategies Lectures on YouTube

Biochemical processes that break things down from larger to smaller are called catabolic processes. Catabolic processes are often oxidative in nature and energy releasing. Some, but not all of that energy is captured as ATP. If not all of the energy is


Sunlight
Photosynthesis
ATP, NADPH
Light
Dark
Anabolism
DNA/RNA, Carbohydrates, Proteins, Fats
Glucose
Catabolism
CO2 NADH, ATP

Biological Energy Movement


Anabolic Versus Catabolic Processes

Nutrients and products of catabolism
Requires reducing agents and energy
Products of anabolism include proteins, nucleic acids, fats, and carbohydrates
Excretion
To catabolism
Anabolism
Reductive
Energy-requiring
Catabolism
Oxidative
Energy-releasing
Fats
Polysaccharides
Proteins
Fatty acids and glycerol
Glucose and other sugars
Amino acids
Smaller molecules
To synthesis of proteins
Excretion
To anabolism

captured as ATP, what happens to the rest of it? The answer is simple. It is released as heat and it is for this reason that we get hot when we exercise. By contrast, synthesizing large molecules from smaller ones (for example, making proteins from amino acids) is referred to as anabolism. Anabolic processes are often reductive in nature and require energy input. By themselves, they would not occur, as they are reversing oxidation and decreasing entropy (making many small things into a larger one). To overcome this energy 'barrier', cells must expend energy. For example, if one wishes to reduce CO_2 to carbohydrate, energy must be used to do so. Plants do this during the dark reactions of photosynthesis. The energy source for the reduction is ultimately the sun. The electrons for the reduction ultimately come from water, and the CO_2 comes from the atmosphere and gets incorporated into a sugar.

Energy Coupling

The addition of phosphate to a sugar is a common reaction that occurs in a cell. By itself, this process is not very energetically favorable (that is, it needs an input of energy to occur). Cells overcome this energy obstacle by using ATP to "drive" the reaction. The energy needed to drive reactions is harvested in very controlled conditions in the confines of an enzyme. This involves a process called 'coupling'. In coupled reactions, an enzyme binds both a high energy molecule (usually ATP) and the other molecule(s) involved in the reaction. Hydrolysis of ATP provides energy for the enzyme to stimulate the reaction on the other substance(s). Hexokinase, for example, catalyzes the phosphorylation of glucose to form

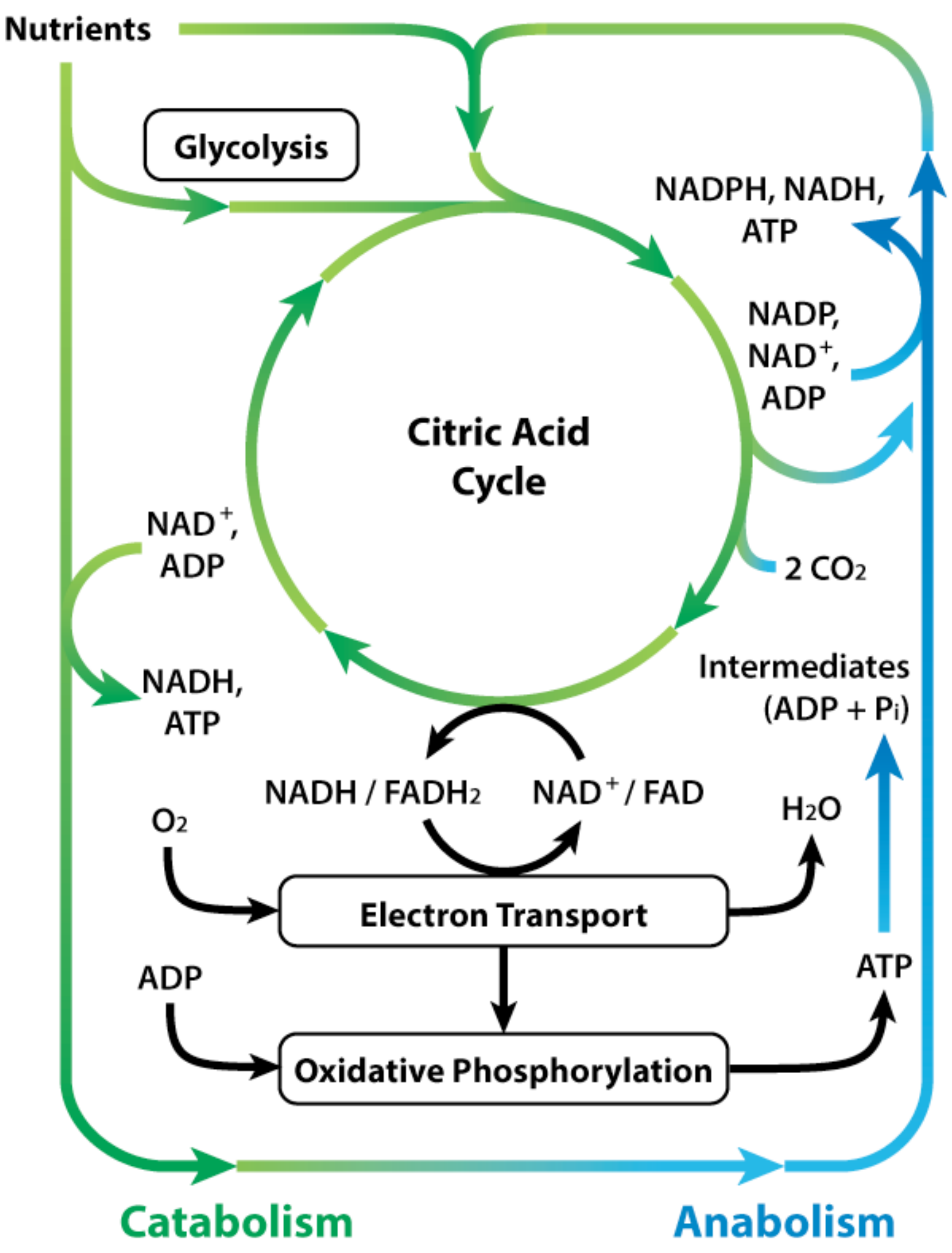
One reason we need ATP
Is the high cost of living, you see
'Cause the chaotic entity
Known as the entropy
Requires cells to burn energy

glucose-6-phosphate. In the absence of ATP, the reaction has a fairly positive ΔG°' (described later), but hydrolysis of ATP provides excess energy, giving the coupled reaction a fairly negative ΔG°' value.

Entropy and Energy

Most students who have had some chemistry know about the principle of the Second Law of Thermodynamics with respect to increasing disorder of a system. Cells are very organized or ordered structures, leading some to mistakenly conclude that life somehow violates the second law. In fact, that notion is incorrect. The second law doesn't say that entropy always increases, just that, left alone, it tends to do so, in an isolated system. Cells are not isolated systems, in that they obtain energy, either from the sun, if they are autotrophic, or food, if they are heterotrophic.

To counter the universal tendency towards disorder on a local scale requires energy. As an example, take a fresh deck of cards which is neatly aligned with Ace-King-Queen . . . . 4,3,2 for each suit. Throw the deck into the air, letting the cards scatter. When you pick them up, they will be more disordered than when they started. However, if you spend a few minutes (and expend a bit of energy), you can reorganize the same deck back to its previous, organized state. If entropy always increased everywhere, you could not do this. However, with the input of energy, you overcame the disorder. The cost of fighting disorder is energy.



There are, of course, other reasons that organisms need energy. Muscular contraction, synthesis of molecules, neurotransmission, signaling, thermoregulation, and subcellular movements are examples. Where does this energy come from? The currencies of energy are generally high-energy phosphate-containing molecules. ATP is the best known and most abundant, but GTP is also an important energy source (required for protein synthesis). CTP is involved in synthesis of glycerophospholipids and UTP is used for synthesis of glycogen. In each of these cases, the energy is in the form of potential chemical energy stored in the multi-phosphate bonds. Hydrolyzing those bonds releases the energy in them.

Of the triphosphates, ATP is the primary energy source, acting to facilitate the synthesis of the others by action of the enzyme NDPK. ATP is made by three distinct types of phosphorylation – oxidative phosphorylation (in mitochondria), photophosphorylation (in chloroplasts of plants), and substrate level phosphorylation (in enzymatically catalyzed reactions).

Oh Delta G

To the tune of "*Danny Boy*"

Oh Delta G - the change in Gibbs free energy
Can tell us if a process will advance
'Cause if the value's less than naught it translates that
Reverse reactions haven't got a chance

But when the sign is plus it is the opposite
And then the backwards happens all the time
A factor is the standard Gibbs free energy
So don't forget about the Delta G naught prime

Recording by Tim Karplus
Lyrics by Kevin Ahern

Gibbs Free Energy

Most of the time, ATP is the "storage battery" of cells (See also 'Molecular Battery Backups for Muscles below). In order to understand how energy is captured, we must first understand Gibbs free energy and in doing so, we begin to see the role of energy in determining the directions chemical reactions take.

Wikipedia defines Gibbs free energy as "a thermodynamic potential that measures the "useful" or process-initiating work obtainable from an isothermal, isobaric thermodynamic system," and further points out that it is "the maximum amount of non-expansion work that can be extracted from a closed system; this maximum can be attained only in a completely reversible process."

Mathematically, the Gibbs free energy is given as

$$G = H - TS$$

where H is the enthalpy, T is the temperature in Kelvin, and S is the entropy.

At standard temperature and pressure, every system seeks to achieve a minimum of free energy. Thus, increasing entropy will reduce Gibbs free energy. Similarly, if excess heat is available (reducing the enthalpy), the free energy can also be reduced. Cells must work within the laws of thermodynamics, as noted, so all of their biochemical reactions, too, have limitations. Now we shall consider energy in the cell. The change in Gibbs free energy (ΔG) for a reaction is crucial, for it, and it alone, determines whether or not a reaction goes forward.

$$\Delta G = \Delta H - T\Delta S,$$

There are three cases

$\Delta G < 0$ - the reaction proceeds as written
$\Delta G = 0$ - the reaction is at equilibrium
$\Delta G > 0$ - the reaction runs in reverse

For a reaction aA <=> bB (where ‘a’ and ‘b’ are integers and A and B are molecules) at pH 7, ΔG can be determined by the following equation,

$$\Delta G = \Delta G^{\circ\prime} + RT\ln([B]^b/[A]^a)$$

For multiple substrate reactions, such as aA + cC <=> bB + dD

$$\Delta G = \Delta G^{\circ\prime} + RT\ln\{([B]^b[D]^d)/([A]^a[C]^c)\}$$

The $\Delta G^{\circ\prime}$ term is called the change in Standard Gibbs Free energy, which is the change in energy that occurs when all of the products and reactants are at standard conditions and the pH is 7.0. It is a constant for a given reaction.

In simple terms, if we collect all of the terms of the numerator together and call them {Products} and all of the terms of the denominator together and call them {Reactants},

$$\Delta G = \Delta G^{\circ\prime} + RT\ln(\{Products\}/\{Reactants\})$$

For most biological systems, the temperature, T, is a constant for a given reaction. Since $\Delta G^{\circ\prime}$ is also a constant for a given reaction, the ΔG is changed almost exclusively as the ratio of {Products}/{Reactants} changes. If one starts out at standard conditions, where everything except protons is at 1M, the RTln({Products}/{Reactants}) term is zero, so the $\Delta G^{\circ\prime}$ term determines the direction the reaction will take. This is why people say that a negative $\Delta G^{\circ\prime}$ indicates an energetically favorable reaction, whereas a positive $\Delta G^{\circ\prime}$ corresponds to an unfavorable one.

When reactions have product largesse
They will act to address the excess
Henry Le Chatelier
Showed conversion’s the way
To suppress the excess by redress

Increasing the ratio of {Products}/{Reactants} causes the value of the natural log (ln) term to become more positive (less negative), thus making the value of ΔG more positive. Conversely, as the ratio of {Products}/{Reactants} decreases, the value of the natural log term becomes less positive (more negative), thus making the value of ΔG more negative.

Intuitively, this makes sense and is consistent with Le Chatelier’s Principle – a system responds to stress by acting to alleviate the stress. If we examine the ΔG for a reaction in a closed system, we see that it will always move to a value of zero (equilibrium), no matter whether it starts with a positive or negative value.

Another type of free energy available to cells is that generated by electrical potential. For example, mitochondria and chloroplasts partly use Coulombic energy (based on charge) from a proton gradient across their membranes to provide the necessary energy for the synthesis of ATP. Similar energies drive the transmission of nerve signals (differential distribution of sodium and potassium) and the movement of some molecules in secondary active transport processes across membranes (e.g., H^+ differential driving the movement of lactose). From the Gibbs free energy change equation,

$$\Delta G = \Delta H - T\Delta S$$

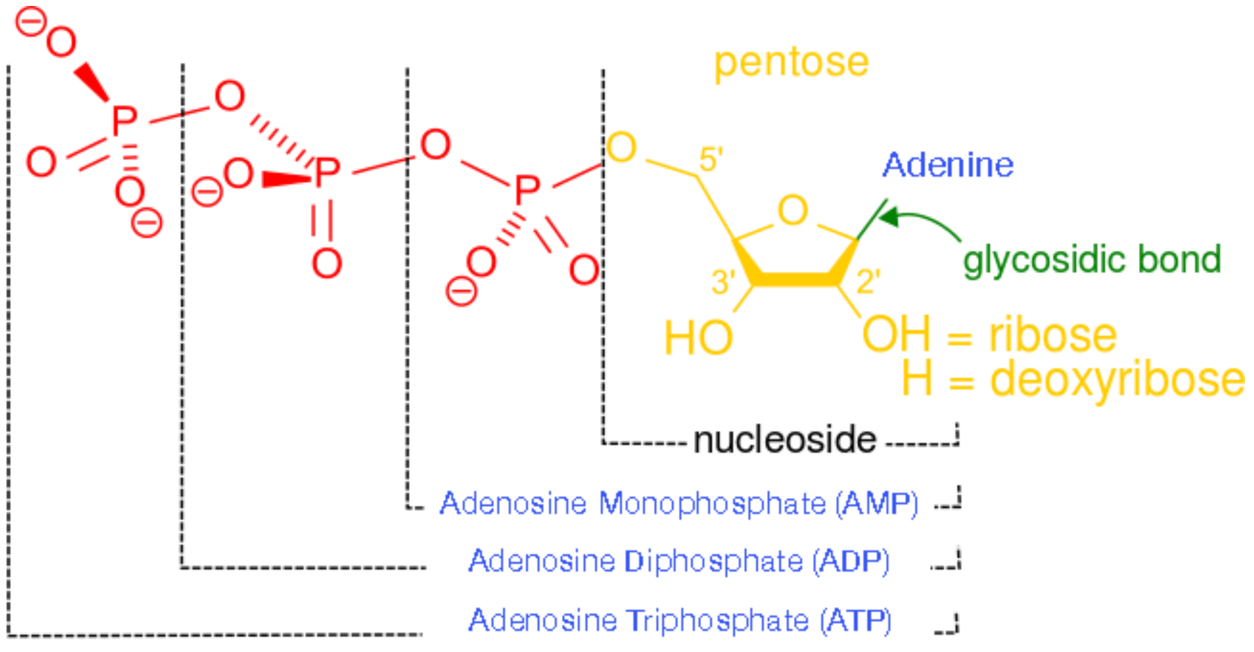
it should be noted that an increase in entropy will help contribute to a decrease in ΔG. This happens, for example when a large molecule is being broken into smaller pieces or when the rearrangement of a molecule increases the disorder of molecules around it. The latter situation arises in the hydrophobic effect, which helps drive the folding of proteins.

Cellular Phosphorylations

Formation of triphosphates is essential to meet the cell's immediate energy needs for synthesis, motion, and signaling. In a given day, an average human being uses more than their body weight in triphosphates. Since triphosphates are the "currency" that meet immediate needs of the cell, it is important to understand how triphosphates are made. There are three phosphorylation mechanisms – 1) substrate level; 2) oxidative; and 3) photophosphorylation. We consider them here individually.

Substrate Level Phosphorylation

The easiest type of phosphorylation to understand is that which occurs at the substrate level. This type of phosphorylation involves the direct synthesis of ATP from ADP and a reactive intermediate, typically a high energy phosphate-containing molecule. Substrate level phosphorylation is a relatively minor contributor to the total synthesis of triphosphates by cells. An example substrate phosphorylation comes from glycolysis.


pentose
Adenine
glycosidic bond
5'
3'
2'
HO
OH = ribose
H = deoxyribose
nucleoside
Adenosine Monophosphate (AMP)
Adenosine Diphosphate (ADP)
Adenosine Triphosphate (ATP)

Adenine Nucleotides

Phosphoenolpyruvate (PEP) + ADP <=> Pyuvate + ATP

This reaction has a very negative ΔG°' (-31.4 kJ/mol), indicating that the PEP contains more energy than ATP, thus energetically favoring ATP's synthesis. Other triphosphates can be made by substrate level phosphorylation, as well. For example, GTP can be synthesized by the following citric acid cycle reaction

Succinyl-CoA + GDP + Pi <=> Succinate + GTP + CoA-SH

Triphosphates can be interchanged readily in substrate level phosphorylations catalyzed by the enzyme Nucleoside Diphosphate Kinase (NDPK). A generalized form of the reactions catalyzed by this enzyme is as follows:

XTP + YDP <=> XDP + YTP

Where X = adenosine, cytidine, uridine, thymidine, or guanosine and Y can be any of these as well. Last, an unusual way of synthesizing ATP by substrate level phosphorylation is that catalyzed by adenylate kinase

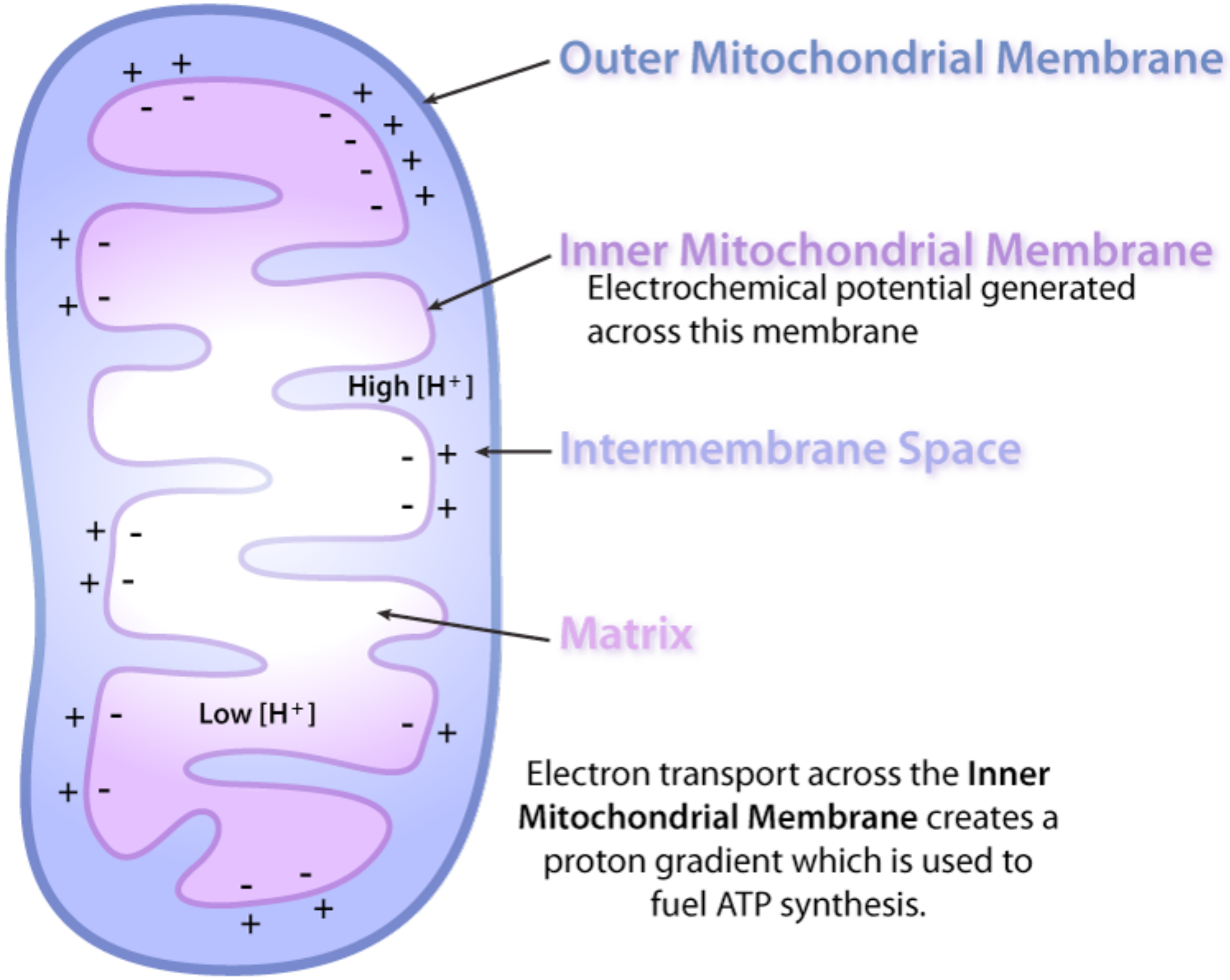
2 ADP <=> ATP + AMP

This reaction is an important means of generating ATP when the cell doesn't have other sources of energy. Accumulation of AMP resulting from this reaction activates enzymes, such as phosphofructokinase, of glycolysis, that will catalyze reactions to give the cell additional, needed energy.

Electron Transport / Oxidative Phosphorylation

Mitocohondria are called the power plants of the cell because most of a cell's ATP is produced there, in a process referred to as oxidative phosphorylation. The mechanism by which ATP is made in oxidative phosphorylation is one of the most interesting processes in all of biology. It has three primary considerations. The first is electrical – electrons from

Click HERE, HERE, and HERE for Kevin's Energy Lectures on YouTube


Outer Mitochondrial Membrane
Inner Mitochondrial Membrane
Electrochemical potential generated across this membrane
High [H⁺]
Intermembrane Space
Matrix
Low [H⁺]

Electron transport across the **Inner Mitochondrial Membrane** creates a proton gradient which is used to fuel ATP synthesis.

I’m a Little Mitochondrion

To the tune of “*I’m a Lumberjack*”

I'm a little mitochondrion
Who gives you energy
I use my proton gradient
To make the ATPs

He's a little mitochondrion
Who gives us energy
He uses proton gradients
To make some ATPs

Electrons flow through Complex II
To traffic cop Co-Q
Whenever they arrive there in
An FADH-two

Electrons flow through Complex II
To traffic cop Co-Q
Whenever they arrive there in
An FADH-two

Tightly coupled is my state
Unless I get a hole
Created in my membrane by
Some di-ni-tro-phe-nol

Yes tightly coupled is his state
Unless he gets a hole
Created in his membrane by
Some di-ni-tro-phenol

Both rotenone and cyanide
Stop my electron flow
And halt the calculation of
My "P" to "O" ratio

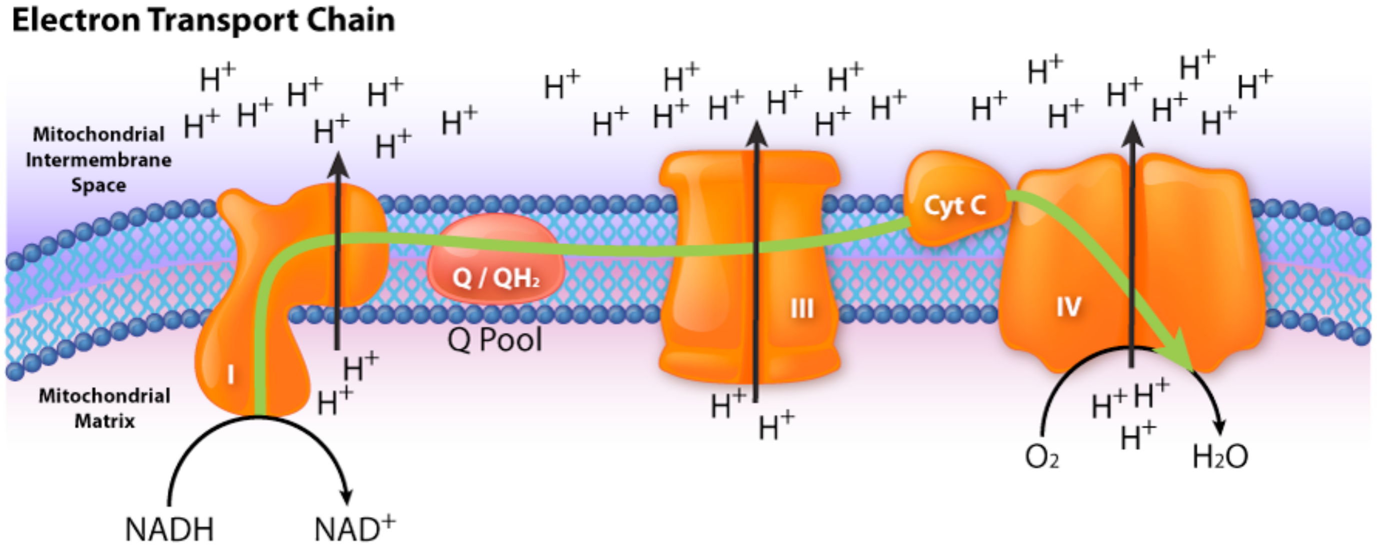
Both rotenone and cyanide
Stop his electron flow
And halt the calculation of
His "P" to "O" ratio

Recording by Tim Karplus
Lyrics by Kevin Ahern

reduced energy carriers, such as NADH and $FADH_2$, enter an electron transport system *via* protein complexes containing iron. As seen in the figure on the following page, electrons move from one complex to the next, not unlike the way they might move through an electrical circuit.

The next consideration arises as a secondary phenomenon. When electrons pass through complexes I, III, and IV, protons are moved from the mitochondrial matrix (inside of mitochondrion) and deposited in the intermembrane space (between the inner and outer membranes of the mitochondrion). The effect of this redistribution is to increase the electrical and chemical potential across the membrane. Students may think of the process as “charging the battery.”

Just like a charged battery, the potential arising from the proton differential across the membrane can be used to do things. This is the third consideration. In the mitochondrion, the “thing” that the proton gradient does is create ATP from ADP and Pi (inorganic phosphate). This process requires energy and is accomplished by movement of protons through a protein complex in the inner mitochondrial membrane. The protein complex is an enzyme that has several names, including Complex V, PTAS (Proton Translocating ATP Synthase), and ATP Synthase. Central to its function is the movement of protons through it (from outside back into the matrix). Protons will *only* move through ATP Synthase if their concentration is greater outside the inner membrane than in the matrix.


Electron Transport Chain
Mitochondrial Intermembrane Space
Mitochondrial Matrix
H^+
I
Q / QH_2
Q Pool
III
Cyt C
IV
O_2
H_2O
NADH
NAD^+

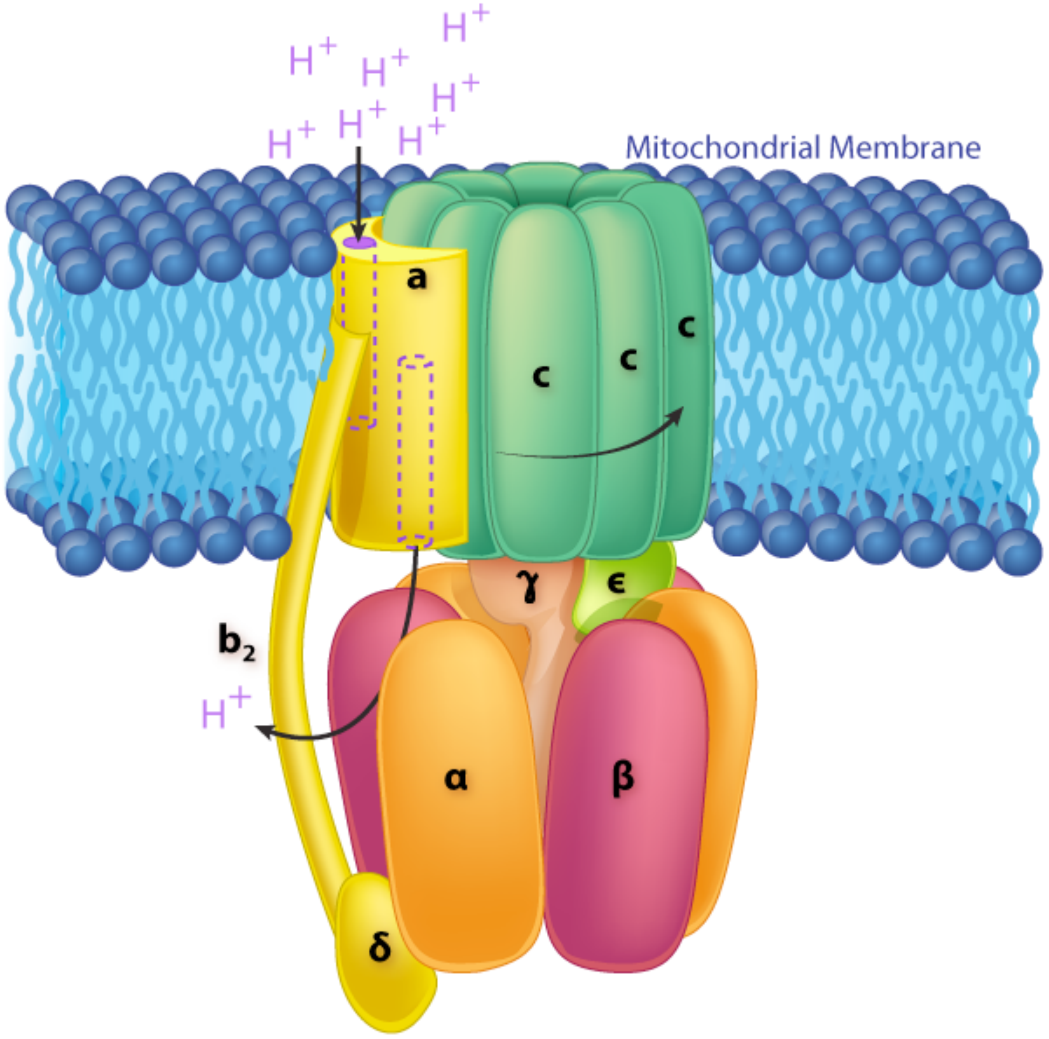
Electron Transport Starting With Complex I

In summary, the electron transport system charges the battery for oxidative phosphorylation by pumping protons out of the mitochondrion. The intact inner membrane of the mitochondrion keeps the protons out, except for those that re-enter through ATP Synthase. The ATP Synthase allows protons to re-enter the mitochondrial matrix and harvests their energy to make ATP.

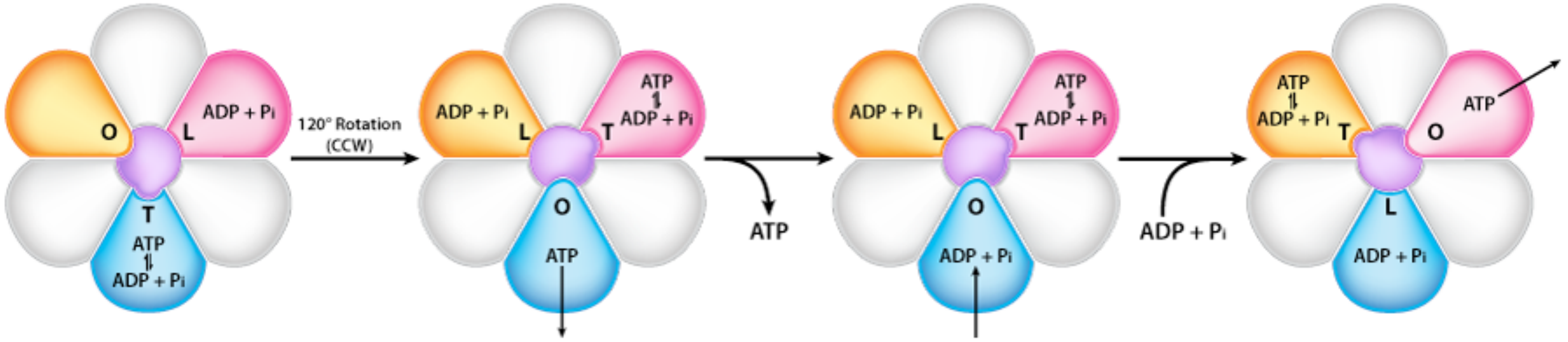
ATP Synthase

The ATP Synthase itself is an amazing nanomachine that makes ATP using a gradient of protons flowing through it from the intermembrane space back into the matrix. It is not easy to depict in a single image what the synthase does. The figure at the right illustrates the multi-subunit nature of this membrane protein, which acts like a turbine at a hydroelectric dam. The movement of protons through the ATP Synthase causes it to spin like a turbine, and the spinning is necessary for making ATP.

In ATP Synthase, the spinning component is the membrane portion (c ring) of the F_0 stalk. The c ring proteins are linked to the gamma-epsilon stalk, which projects into the F_1 head of the mushroom structure. The F_1 head contains the catalytic ability to make ATP.


H^+
Mitochondrial Membrane
a
c
c
c
γ
ϵ
b_2
H^+
α
β
δ

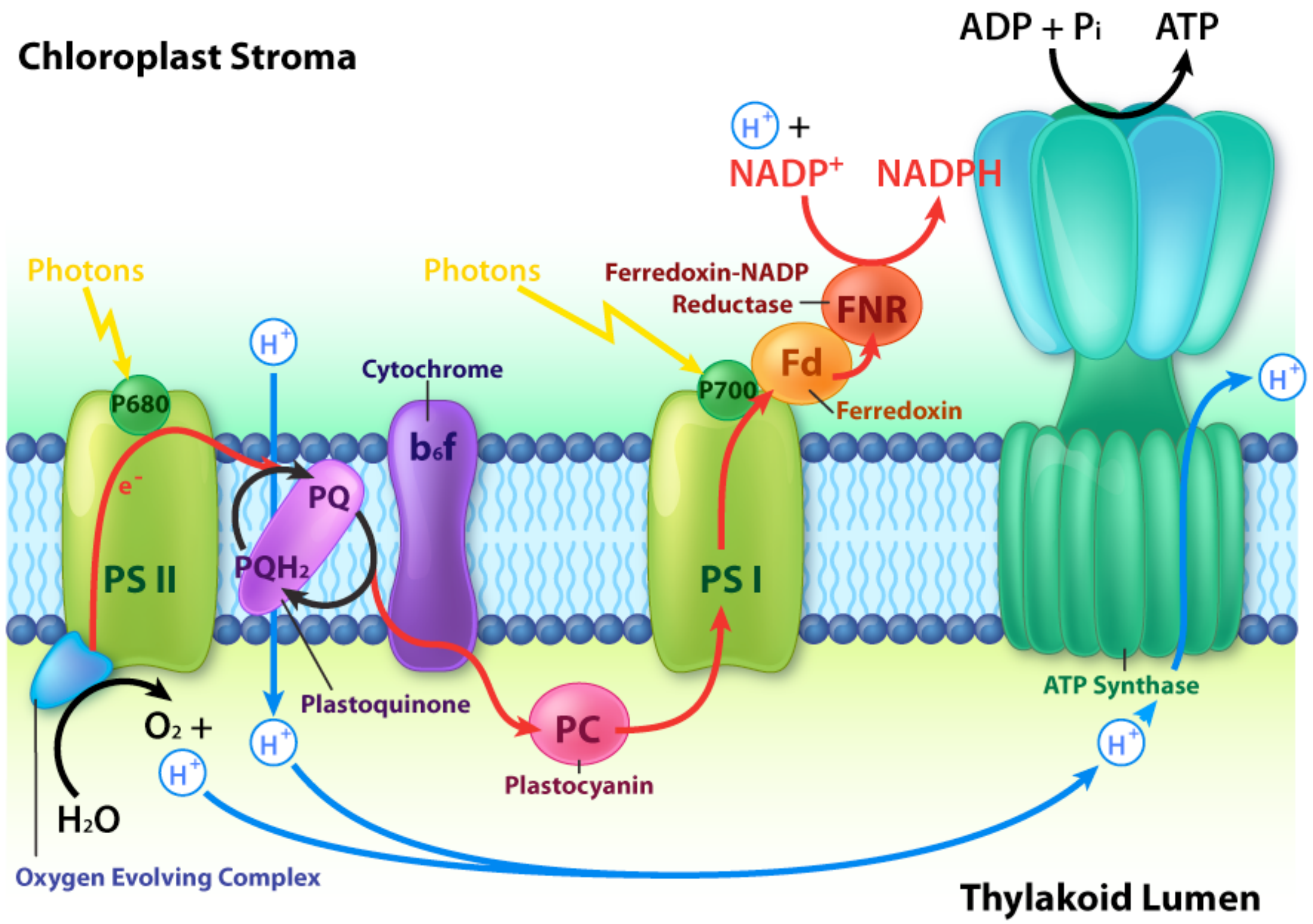
ATP Synthase (Complex V)


O
L
ADP + Pi
T
ATP ⇅ ADP + Pi
120° Rotation (CCW)
ADP + Pi
L
ATP ⇅ ADP + Pi
T
O
ATP
ATP
ADP + Pi
L
ATP ⇅ ADP + Pi
T
O
ADP + Pi
ADP + Pi
ATP ⇅ ADP + Pi
T
ATP
O
L
ADP + Pi

Three States of ATP Synthase

Click HERE for Kevin's Photosynthesis Lecture on YouTube

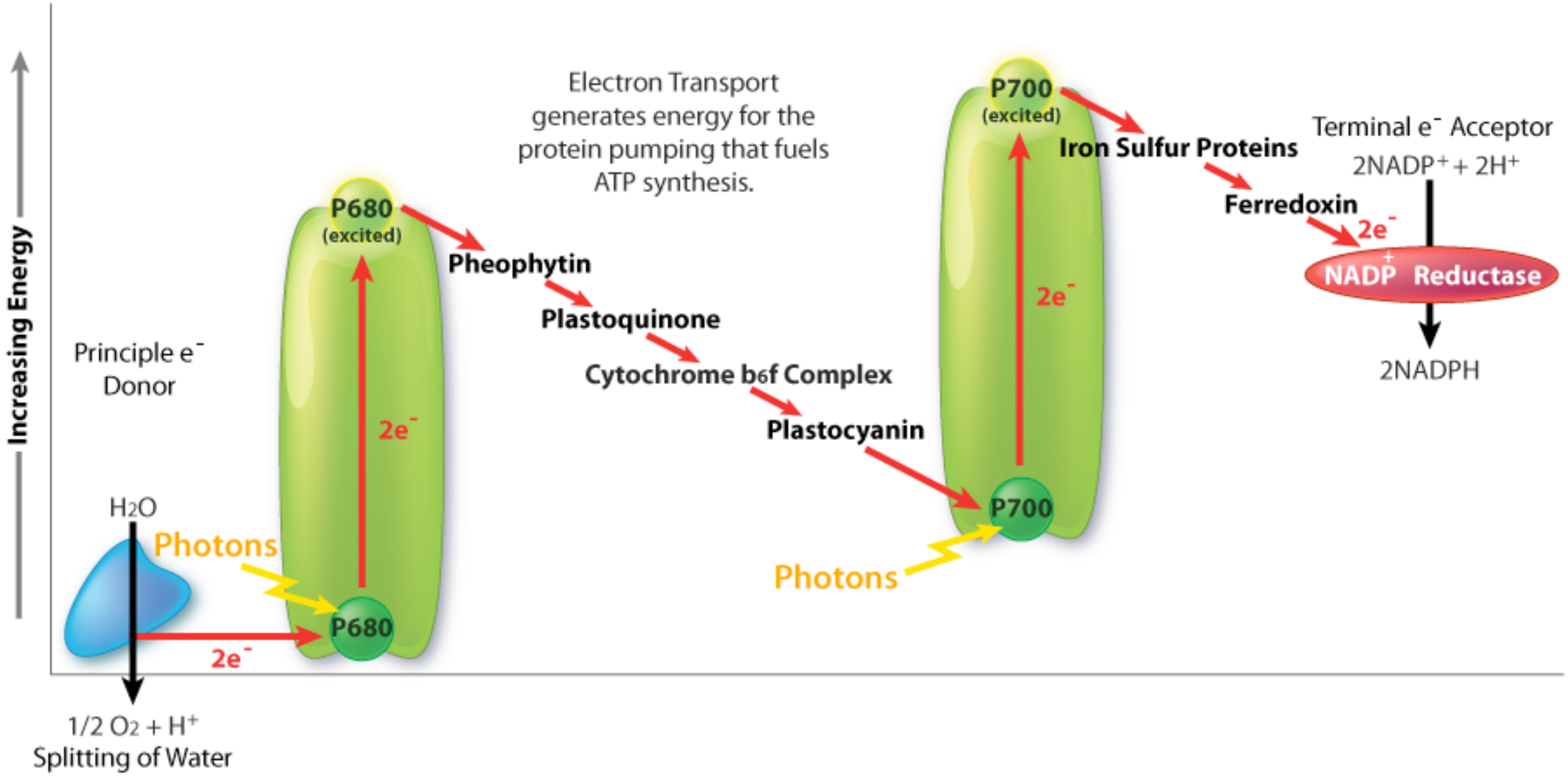
The F_1 head is hexameric in structure with paired alpha and beta proteins arranged in a trimer of dimers. Movement of the gamma protein inside the alpha-beta trimer causes each set of beta proteins to change structure slightly into three different forms called Loose, Tight, and Open (L,T,O). Each of these forms has a function. The Loose form binds ADP + P_i. The tight form "squeezes" them together to form the ATP. The open form releases the ATP into the mitochondrial matrix. Thus, as a result of the proton excess in the intermembrane space, ATP is made.


Chloroplast Stroma
ADP + Pi
ATP
H+ +
NADP+
NADPH
Photons
Photons
Ferredoxin-NADP Reductase
FNR
Fd
H+
Cytochrome
P700
P680
Ferredoxin
b6f
H+
PQ
e-
PS II
PQH2
PS I
Plastoquinone
PC
ATP Synthase
O2 +
H+
H+
H+
H2O
Plastocyanin
Oxygen Evolving Complex
Thylakoid Lumen

Photophosphorylation

Photophosphorylation

The third type of phosphorylation to make ATP is found only in cells that carry out photosynthesis. This process is similar to oxidative phosphorylation in several ways. A primary difference is the ultimate source of the energy for ATP synthesis. In oxidative phosphorylation, the energy comes from electrons produced by oxidation of biological molecules. In the case of photosynthesis, the energy comes from the light of the sun.


Increasing Energy
Electron Transport generates energy for the protein pumping that fuels ATP synthesis.
Principle e⁻ Donor
H₂O
Photons
2e⁻
1/2 O₂ + H⁺
Splitting of Water
P680
P680 (excited)
2e⁻
Pheophytin
Plastoquinone
Cytochrome b6f Complex
Plastocyanin
Photons
P700
2e⁻
P700 (excited)
Iron Sulfur Proteins
Ferredoxin
2e⁻
Terminal e⁻ Acceptor
2NADP⁺ + 2H⁺
NADP⁺ Reductase
2NADPH

Electron Movement in Photosynthesis

Photons from the sun interact with chlorophyll molecules in reaction centers in the chloroplasts of plants or membranes of photosynthetic bacteria. A schematic of the process is shown above.

The similarities of photophosphorylation to oxidative phosphorylation include:

- an electron transport chain
- creation of a proton gradient
- harvesting energy of the proton gradient by making ATP with the help of an ATP synthase.

Some of the differences include :

- the source of the electrons – H_2O for photosynthesis versus NADH/ $FADH_2$ for oxidative phosphorylation
- direction of proton pumping – into the thylakoid space of the chloroplasts versus outside the matrix of the mitochondrion
- movement of protons during ATP synthesis – out of the thylakoid space in photosynthesis versus into the mitochondrial matrix
- nature of the terminal electron acceptor – $NADP^+$ in photosynthesis versus O_2 in oxidative phosphorylation.

Electron Transport in Chloroplasts versus Mitochondria

In some ways, the movement of electrons in chloroplasts during photosynthesis is opposite that of electron transport in mitochondria. In photosynthesis, water is the source of electrons and their final destination is NADPH. In mitochondria, NADH/$FADH_2$ are electron sources and H_2O is their final destination. How do biological systems get electrons to go both ways? It would seem to be the equivalent of going to and from a particular place while always going downhill, since electrons will move according to potential. The answer is the captured energy of the photons, which elevates electrons in photosynthesis to an energy where they move “downhill” to their NADPH destination in a Z-shaped scheme (previous page). The movement of electrons through this scheme in plants requires energy from photons in two places to “lift” the energy of the electrons sufficiently.

N-A-D

To the tune of “*Penny Lane*”

In the catabolic pathways that our cells employ
Oxidations help create the ATP
While they lower Gibbs free energy
Thanks to enthalpy

If a substrate is converted from an alcohol
To an aldehyde or ketone it is clear
Those electrons do not disappear
They just rearrange – very strange

N-A-D is in my ears and in my eyes
Help-ing mol-e-cules get oxidized
Making N-A-D-H then

And the latter is a problem anaerobically
‘Cuz accumulations of it muscles hate
They respond by using pyruvate
To produce lactate

Catalyzing is essential for the cells to live
So the enzymes grab their substrates eagerly
If they bind with high affinity
Low K_m you see, just as me

N-A-D is in my ears and in my eyes
Help-ing mol-e-cules get oxidized
Making N-A-D-H then

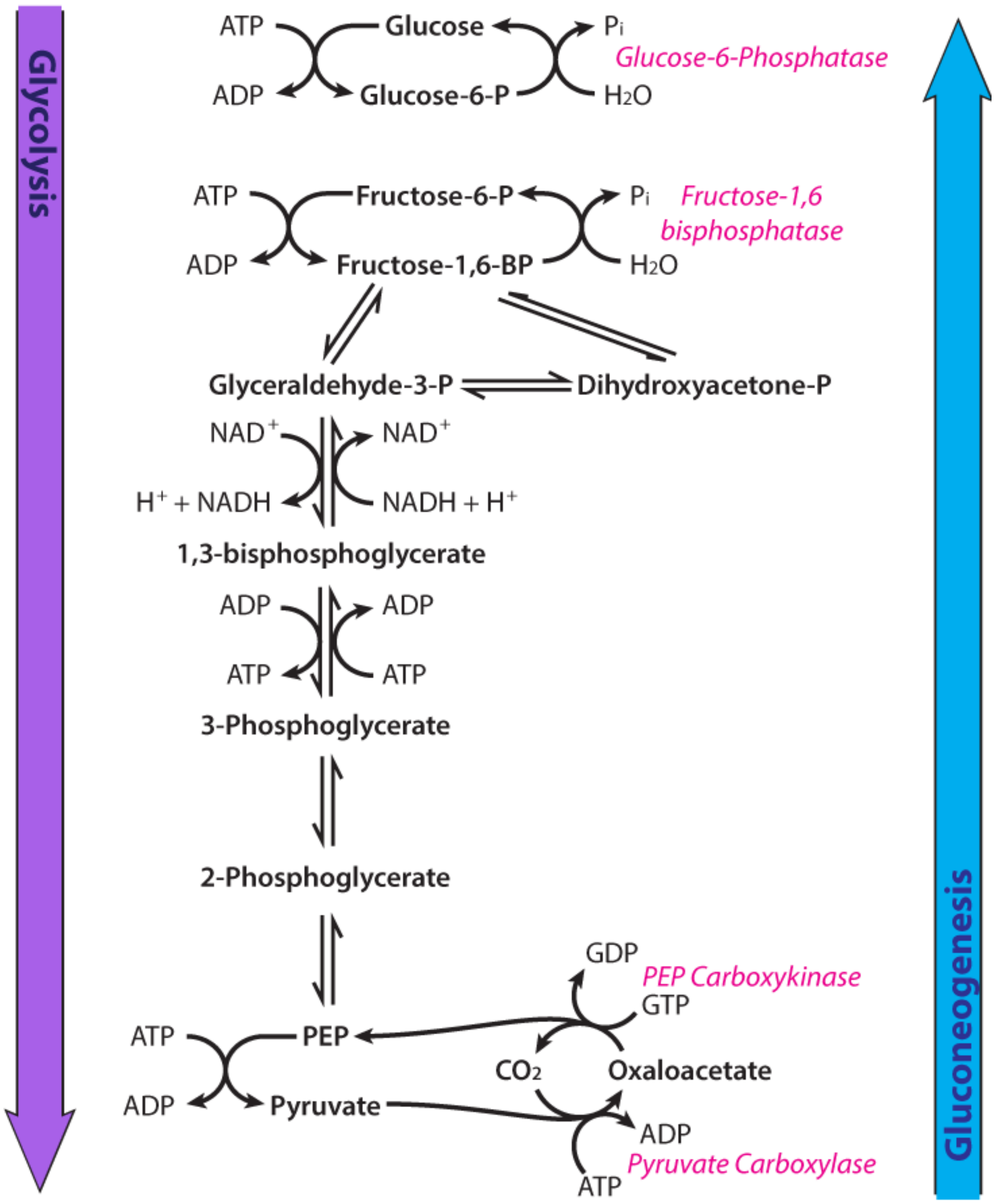
Recorded by Tim Karplus
Lyrics by Kevin Ahern

Photosynthesis left me aghast
As electrons all went whizzing past
Leaving water at start
They trace the Z chart
In the membranes of each chloroplast

Last, it should be noted that photosynthesis actually has two phases, referred to as the light cycle (described above) and the dark cycle, which is a set of chemical reactions that captures CO_2 from the atmosphere and “fixes” it, ultimately into glucose. The dark cycle is also referred to as the Calvin Cycle and is discussed HERE.

Energy Efficiency

Cells are not 100% efficient in energy use. Nothing that we know of is. Consequently, cells do not get as much energy out of catabolic processes as they put into anabolic processes. A good example is the synthesis and breakdown of glucose, something liver cells are frequently doing. The complete conversion of glucose to pyruvate in glycolysis (catabolism) yields two pyruvates plus 2 NADH plus 2 ATPs. Conversely, the complete conversion of two pyruvates into glucose by gluconeogenesis (anabolism) requires 4 ATPs, 2 NADH, and 2 GTPs. Since the energy of GTP is essentially equal to that of ATP, gluconeogenesis requires a net of 4 ATPs more than glycolysis yields. This difference must be made up in order for the organism to balance everything. It is for this reason that we eat. In addition, the inefficiency of our capture of energy in reactions results in the production of heat and helps to keep us warm. You can read more about glycolysis and gluconeogenesis HERE.


Glycolysis
ATP
Glucose
Pi
Glucose-6-Phosphatase
ADP
Glucose-6-P
H2O
ATP
Fructose-6-P
Pi
Fructose-1,6 bisphosphatase
ADP
Fructose-1,6-BP
H2O
Glyceraldehyde-3-P
Dihydroxyacetone-P
NAD+
NAD+
H+ + NADH
NADH + H+
1,3-bisphosphoglycerate
ADP
ADP
ATP
ATP
3-Phosphoglycerate
2-Phosphoglycerate
GDP
PEP Carboxykinase
GTP
ATP
PEP
CO2
Oxaloacetate
ADP
Pyruvate
ADP
Pyruvate Carboxylase
ATP
Gluconeogenesis

Glycolysis and Gluconeogenesis

Metabolic Controls of Energy

It is also noteworthy that cells do not usually have both catabolic and anabolic processes for the same molecules (for example, breakdown of glucose and synthesis of glucose, shown on the previous page) occurring simultaneously inside of them because the cell would see no net production of anything but heat and a loss of ATPs with each turn of the cycle. Such cycles are called futile cycles and cells have controls in place to limit the extent to which they occur. Since futile cycles can, in fact, yield heat, they are sources of heat in some types of tissue. See also HERE for more on futile cycles.

Molecular Backups for Muscles

For plants, the needs for energy are different than for animals. Plants do not need to access energy sources as rapidly as animals do, nor do they have to maintain a constant internal temperature. Plants can neither flee predators, nor chase prey. These needs of animals are much more immediate and require that energy stores be accessible on demand. Muscles, of course, enable the motion of animals and the energy required for muscle contraction is ATP. To have stores of energy readily available, muscles have, in addition

The Muscle Energy Song

To the tune of "*I Will*"

For running and for jumping
You need some energy
Chemically the body stores it
In the form of ATP

If backup should be needed
Reserves are there in wait
Muscles brimming with supplies of
Tiny creatine phosphate

Ready whenever you are ever
Wanting to exercise
Steady as ever when whatever
Energy needs arise

The action is exacting
For leaping in the air
Myofibrils all contracted
Using energy extracted

From reactions that react in me
Using A-T-P
You see

Recorded by David Simmons
Lyrics by Kevin Ahern

to ATP, creatine phosphate and glycogen for quick release of glucose from glycogen. The synthesis of creatine phosphate is a prime example of the effects of concentration on the synthesis of high energy molecules. For example, creatine phosphate has an energy of hydrolysis of -43.1 kJ/mol whereas ATP has an energy of hydrolysis of -30.5 kJ/mol Creatine phosphate, however, is made from creatine and ATP in the reaction shown below. How is this possible?

Creatine + ATP <=> Creatine phosphate + ADP

The $\Delta G^{\circ\prime}$ of this reaction is +12.6 kJ/mol, reflecting the energies noted above. In a resting muscle cell, ATP is abundant and ADP is low, driving the reaction to the right, creating creatine phosphate. When muscular contraction commences, ATP levels fall and ADP levels climb. The above reaction then reverses and proceeds to synthesize ATP immediately. Thus creatine phosphate acts like a battery, storing energy when ATP levels are high and releasing it almost instantaneously to create ATP when its levels fall.

Summary

In summary, energy is needed for cells to perform the functions that they must carry out in order to stay alive. At its most basic level, this means fighting a continual battle with entropy, but it is not the only need for energy that cells have.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Chapter 3

Structure & Function

Function flows from structure. In order to understand the function of biomolecules, we must first understand their structures.

Chapter 3

Structure and Function

- Building Blocks
- Proteins
  - Primary Structure
  - Secondary Structure
    - Alpha helix
    - Beta Strands
  - Fibrous Proteins
  - Ramachandran Plots
  - Tertiary Structure
  - Hydrophobic Effects
  - Quaternary Structure
  - Other Protein Structural Features
  - Cooperativity
  - Bohr Effect
  - 2,3 BPG
  - Denaturation
  - Forces Stabilizing Structure
  - Refolding Denatured Proteins
  - Irreversible Denaturation
  - Prions and Misfolding
- Nucleic Acids
  - Superhelicity
  - RNA Structures
  - Denaturing Nucleic Acids
- Carbohydrates
  - Monosaccharides
  - Stereoisomer Nomeclature
  - Boat/Chair Conformations
  - Oligosaccharides
  - Polysaccharides
  - Amylose/Amylopectin
  - Glycogen
  - Cellulose
  - Glycosaminoglycans
- Lipids and Membranes
  - Fatty Acids
  - Membrane Lipids
  - Lipid Bilayers
  - Membrane Proteins
  - Membrane Transport
  - Sodium-Potassium ATPase
  - Bacteriorhodopsin
  - Fat Soluble Vitamins

If we hope to understand function in biological systems, we must first understand structure. At a simple level, we can divide molecules up according to their affinities for water – **hydrophobic** (limited solubility in water), **hydrophilic** (soluble in water) and **amphiphilic** (have characteristics of both hydrophobicity and hydrophilicity). **Hydrophobicity** in biological molecules arises largely because carbon-hydrogen bonds have electrons that are fairly evenly shared (not unlike carbon-carbon bonds). By contrast, the electrons between the oxygen and hydrogen of water are not equally shared. Oxygen has a greater electronegativity, so it holds them closer than hydrogen does. As a consequence, oxygen has what we call a partial negative charge and hydrogen has a partial positive charge.

Virtually all of life on Earth is built upon the biochemistry that arises from the molecular properties described in the preceding paragraph. The biomolecules referred to as lipids are largely water insoluble because they have predominantly carbon-hydrogen bonds with few ionic or hydrogen bond characteristics.

Building Blocks

Click HERE for Kevin's YouTube lecture on amino acids

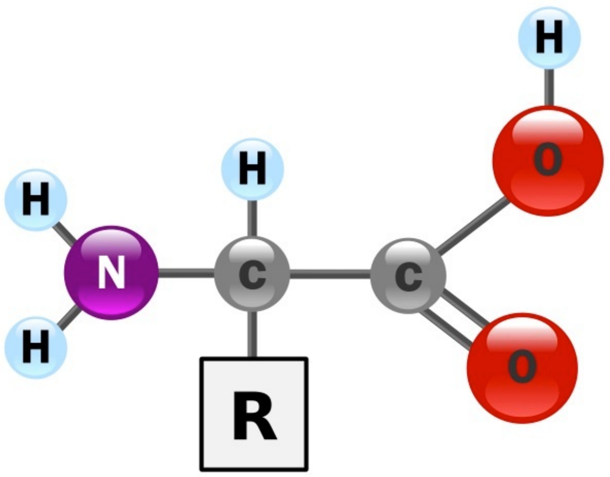
Biological macromolecules are all polymers of a sort, even fats, in which the fatty acids can be thought of as polymers of carbon. (We will consider fatty compounds - **fats**,

glycerophospholipids, **sphingolipids**, **isoprenoids**/ **terpenoids** separately).

The remaining categories of biological macromolecules include **proteins**, **nucleic acids**, and polysaccharides. The building blocks of these, respectively, are amino acids, **nucleotides**, and **monosaccharides** (sugars). Of these, the most diverse collection of chemical properties is found among the amino acids.

Proteins

Whereas nucleotides all are water soluble and have the same basic composition (sugar, base, phosphate) and the sugars also are water soluble and mostly contain 5 or 6 carbons (a few exceptions), the amino acids (general structure below) are structurally and chemically diverse. Though all of the amino acids are, in fact, soluble in water, the interactions of their side chains with water differ significantly. This is important, because it is only in the side chains (R-groups) that amino acids differ from each other. Based



Amino Acid Schematic

from Wikipedia

The Amino Alphabet Song

To the tune of "*Twinkle, Twinkle Little Star*"

Lysine, arginine and his
Basic ones you should not miss
Ala, leu, val, ile, and met
Fill the aliphatic set
Proline bends and cys has 's'
Glycine's 'R' is the smallest
Then there's trp and tyr and phe
Structured aromatically

Asp and glu's side chains of R
Say to protons "au revoir"
Glutamine, asparagine
Bear carboxamide amines
Threonine and tiny ser
Have hydroxyl groups to share
These twen-TY amino A's
Can combine a zillion ways

Recorded by David Simmons
Lyrics by Kevin Ahern

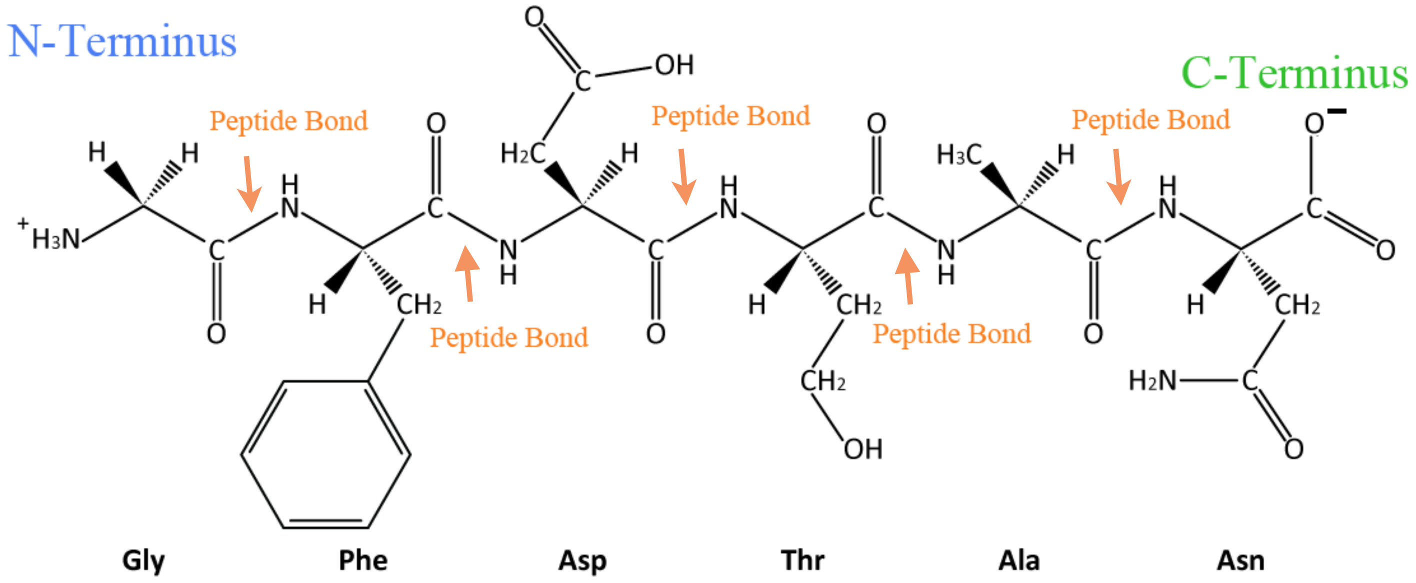
on side chains, we can group the 20 amino acids found in proteins as follows:

- **Aromatic** (**phenylalanine**, **tyrosine**, **tryptophan**)
- **Aliphatic** (**leucine**, **isoleucine**, **alanine**, **methionine**, **valine**)
- **Hydroxyl/Sulfhydryl** (**threonine**, **serine**, tyrosine, **cysteine**)
- **Carboxyamide** (**glutamine**, **asparagine**)
- **R-Acids** (**glutamic acid**, **aspartic acid**)
- **R-Amines** (**lysine**, **histidine**, **arginine**)
- **Odd** (**glycine**, **proline**)

Note that tyrosine has a hydroxyl group and fits into two categories. Note also that biochemistry books vary in how they organize amino acids into categories.

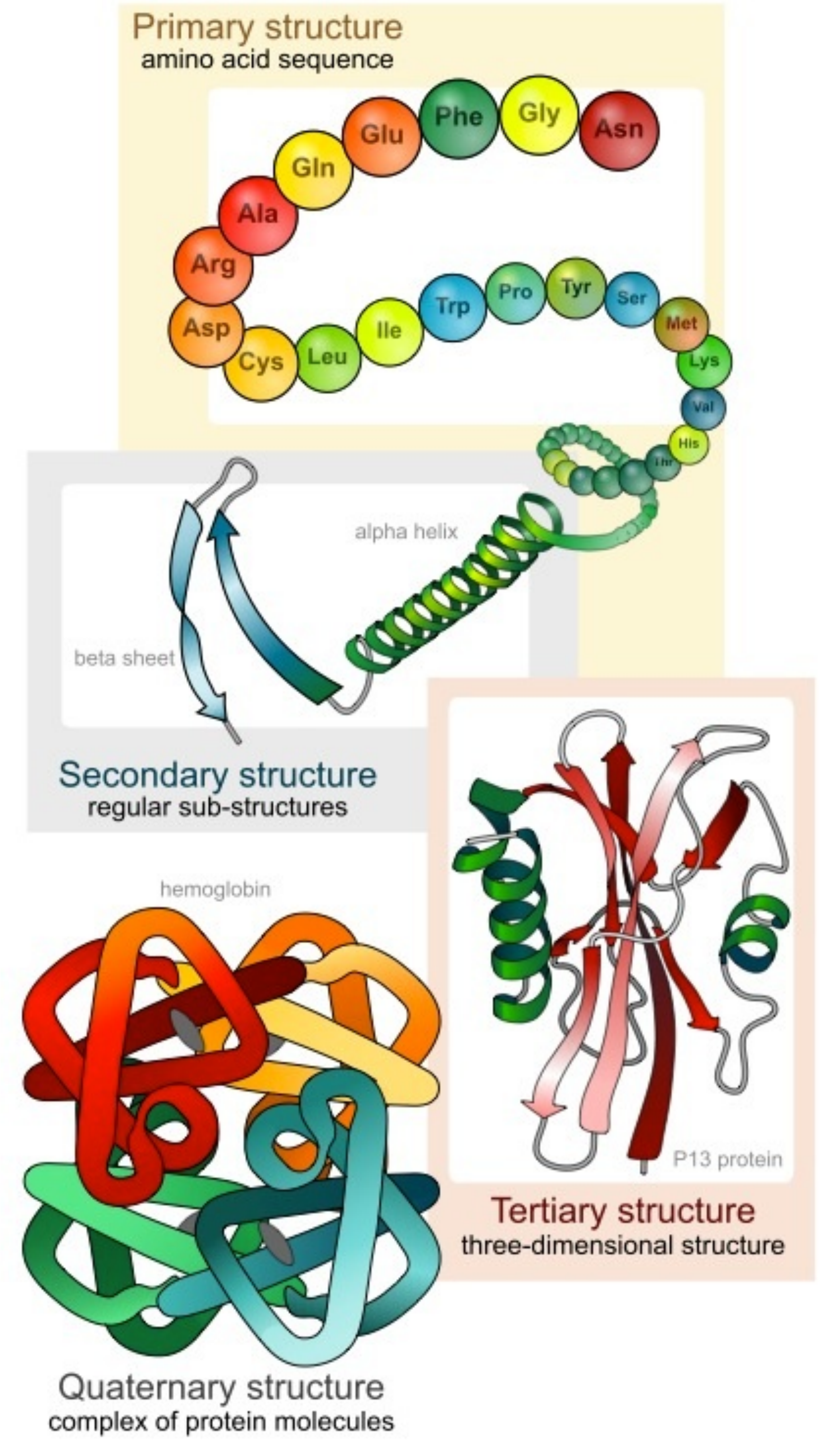
Amino acids are joined to each other by **peptide bonds**. This introduces a slight simplifying aspect to the structure of proteins – one need only consider the positioning of the R-groups around each peptide bond when determining protein structure schematically. Proteins that are in aqueous environments, such as the cytoplasm of the cell, have their amino acids arranged so that those with **hydrophilic side chains** (such as threonine or lysine) predominate on the exterior of the protein so as to interact with water. The **hydrophobic amino acids** in these proteins are found predominantly on the interior. When one examines the structure of proteins in non-aqueous environments, such as the interior of a lipid bilayer, the arrangement is flipped – hydrophobics predominate on the outside where they can interact with the hydrophobic side chains of membrane fatty acids and the hydrophilic amino acids are arranged anyplace where they can contact water. For a protein like **porin**, which provides an interior channel through which water can pass, this is where the hydrophilics are found. For transmembrane proteins, which project through both sides of the membrane, the hydrophilics are found at each point where the polypeptide chain emerges from the membrane.

Click HERE and HERE, for Kevin's Protein Structure lectures on YouTube



Primary Structure

How do proteins obtain such arrangements of amino acids? As we shall see, the structures of all proteins ultimately arise from their amino acid sequences. The amino acid sequence is referred to as the **primary structure** and changes in it can affect every other level of structure as well as the properties of a protein. The primary structure of a protein arrived at its current state as a result of mutation and selection over evolutionary time. On a more immediate time scale, 3D protein structure arises as a result of a phenomenon called **folding**. **Protein folding** results from three different structural elements beyond primary structure. They are referred to as **secondary**, **tertiary**, and quaternary structures, each arising from interactions between progressively more distant amino acids in the primary structure.


Primary structure
amino acid sequence
Glu
Phe
Gly
Asn
Gln
Ala
Arg
Asp
Cys
Leu
Ile
Trp
Pro
Tyr
Ser
Met
Lys
Val
His
Thr
alpha helix
beta sheet
Secondary structure
regular sub-structures
hemoglobin
P13 protein
Tertiary structure
three-dimensional structure
Quaternary structure
complex of protein molecules

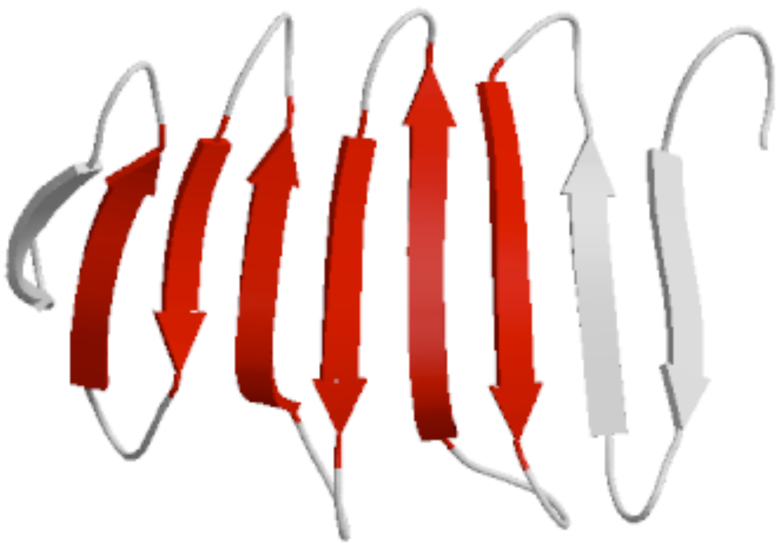
from Wikipedia

Secondary Structure

Interactions between amino acids within about ten units of each other give rise to regular repeating structures. These **secondary structures** include the well known **alpha-helix** and **beta strands**. Both were predicted by **Linus Pauling**, **Robert Corey**, and **Herman Branson** in 1951. Each structure has unique features. We use the terms **rise**, **repeat**, and **pitch** to describe the parameters of a helix. The repeat is the number of residues in a helix before it begins to repeat itself. The rise is the distance the helix elevates with addition of each residue. The pitch is the distance between the turns of the helix.

Alpha Helix

The **alpha helix** (left) forms as a result of interactions between amino acids separated by four residues. Interestingly, the side chains of the amino acids in an alpha helix are all pointed outwards from the axis of the helix. Alpha helices have a repeat of 3.6 amino acid residues per turn of the helix, meaning that four turns of the helix have approximately 14 amino acid residues. Hydrogen bonds occur between the C=O of one amino acid and the N-H of



A Beta Sheet

from Wikipedia

another amino acid four residues distant and these help to stabilize the structure (note that the C=O and N-H involved are part of the polypeptide backbone, not the R-groups) Some amino acids have high helix forming tendencies. They include methionine, alanine, leucine, uncharged glutamate, and lysine. Others, such as proline, glycine, and negatively charged aspartate, disfavor its formation.

Beta Strands

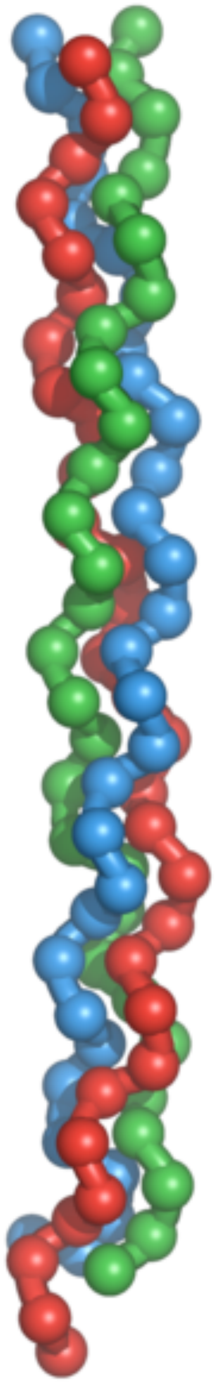
Beta strands are the most fundamental helix, having essentially a 2D backbone of 'folds' like those of the pleats of a curtain. Indeed, beta strands can be arranged together to form what are called **beta sheets**. Other regular structures are also known. What determines whether a given stretch of a protein is in a helical or other structure? Here is where the shape and chemistry of the side chains plays a role.

Fibrous Proteins

Not all proteins have significant amounts of tertiary or quaternary structure. (As we shall see, these last two levels of structure arise from 'bends' in the polypeptide chains and interactions between separate polypeptide chains, respectively.)

Alpha keratin, for example, is what we refer to as a **fibrous protein** (also called **scleroprotein**). Alpha keratin has primary structure and secondary structure, but little tertiary or quaternary structure. Consequently, alpha keratin exists mostly as long fibers, such as are found in hair. **Beta-keratin** is a harder fibrous protein found in nails, scales, and claws. It is made up mostly of

We wouldn't be too popular
If keratins were globular
Our nails and hair would be as knots
Their structures folded up like clots
No strength they'd have and oh by gosh
They'd rearrange with every wash



Collagen

from Wikipedia

Click HERE and HERE, for Kevin's Protein Structure Lectures on YouTube

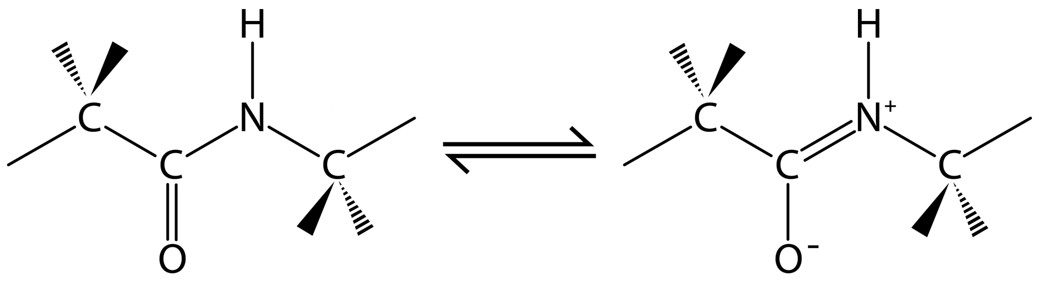
beta sheets. Proline, which is the least flexible amino acid, due to attachment of the side chain to the alpha-amino group, is less likely to be found in alpha helices, but curiously it is found abundantly in the fibrous protein known as collagen. **Collagen** (previous page) is the most abundant protein in the human body and is the 'glue' that literally sticks us together. How does the inflexibility of proline permit it to be in a helix? The answer is probably the parallel abundance in collagen of glycine, which contains the smallest side group and therefore has the greatest flexibility.

An interesting sidelight of the presence of proline in collagen is the chemical modification of prolines, by the addition of hydroxyl groups, after the protein is made. Such '**post-translational modifications**' are not uncommon. Threonine, serine, and tyrosine frequently have their hydroxyl side-chains phosphorylated. Lysines in collagen too are hydroxylated post-translationally. The hydroxylated prolines and lysines play a role in the formation of interchain hydrogen bonds and crosslinking of triple helices during the assembly of collagen fibrils. These bonds provide structural integrity to the collagen. The enzymes that add hydroxyls to proline and lysine require **vitamin C** (**ascorbic acid**) for their activity. Lack of vitamin C leads to the production of weakened collagen fibrils, resulting in a condition called scurvy.

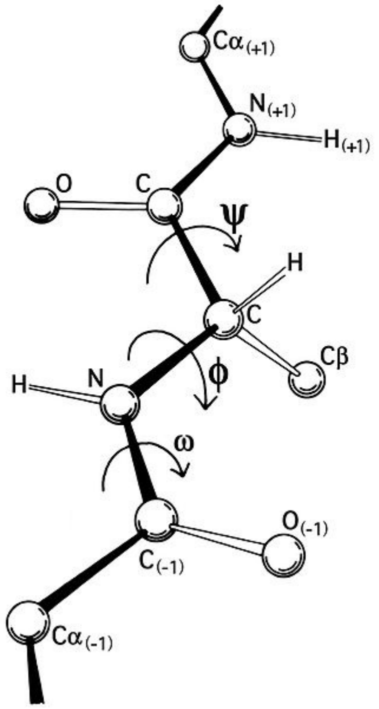
The carbonyl oxygen of the peptide bond can exist in resonance with the C-N bond, giving the peptide bond characteristics of a double bond and imposing limitations for rotation around it. If we treat the peptide bond as a double bond, then the arrangements of adjacent carbon bonds around it can be thought of as being in the *cis* or *trans* configurations. In proteins, not surprisingly, the preferred arrangement of these groups is strongly *trans* (1000/1). Of the 20 amino acids, the one that favors peptide bonds in the *cis* configuration most commonly is proline, but even for proline, the *trans* isomer is strongly preferred.

Ramachandran Plots

Another consequence of considering the peptide bond as a double bond is that it reduces the number of variable rotational angles of the polypeptide backbone. The terms **phi** and **psi** refer to rotational angles about the bonds between the N-alpha carbon



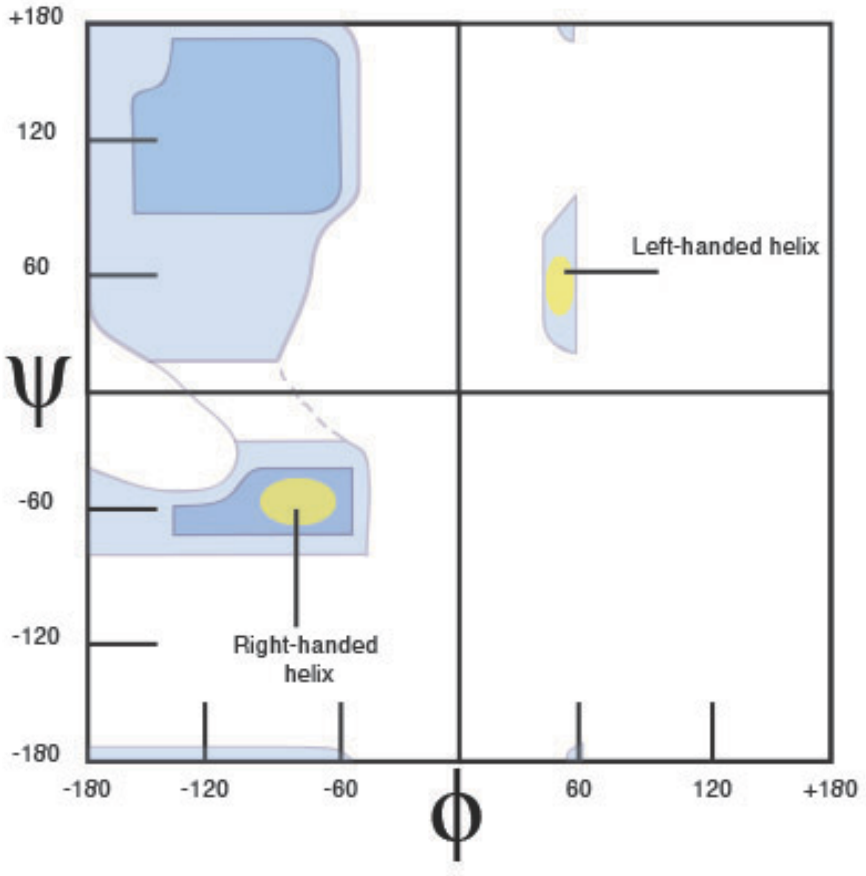
Peptide Bond Resonance



Phi and Psi Angles

from Wikipedia

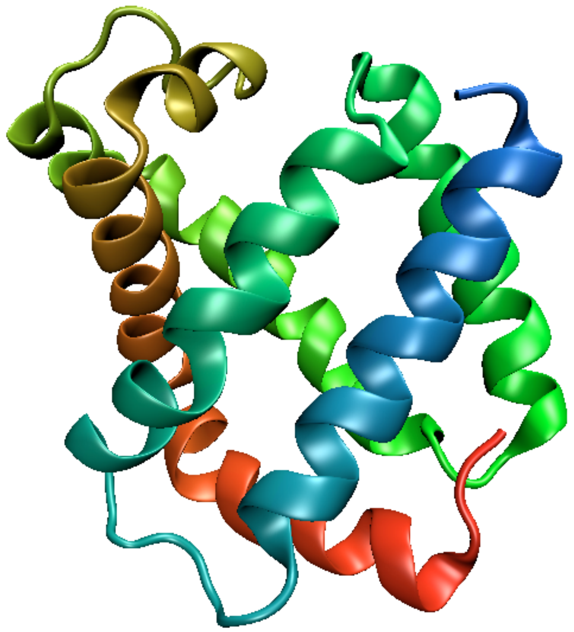
and alpha carbon-carbonyl carbon respectively (previous page). Given the bulkiness of R-groups, the phenomenon of **steric hindrance** and the tendency of close side chains to interact with each other, one might expect to find a bias in the values of phi and psi. Indeed, that is exactly what is observed. Dr. G.N. **Ramachandran** proposed such a result and, in a plot that bears his name, depicted the theoretical likelihood of each angle appearing in a polypeptide. More recent observations of actual phi and psi angles in data from the PDB protein database bear out Dr. Ramachandran's predictions. In the plot above, beta strands fit nicely in the darker blue section at the top and alpha helices fit in the yellow section near the middle.



Ramachandran Plot

Tertiary Structure

In contrast to secondary structures, which arise from interactions between amino acids close in primary structure, **tertiary structure** arises from interactions between amino acids more distant in primary structure. Such interactions are not possible in an endlessly stretching fiber because each amino acid placed between two amino acids causes them to be moved farther away from each other in what is essentially the two dimensions of a secondary structure. For distant amino acids to interact, they must be brought into closer proximity and this



The Structure of Myoglobin

from Wikipedia

requires bending and folding of the polypeptide chain. Proteins with such structures are referred to as ‘globular’ and they are, by far, the most abundant class of proteins. Indeed, it is in **globular proteins** that we have the most vivid images of the results of folding.

“Folds” in **polypeptides** arise as a result of ‘**bends**’ between regions of secondary structure (such as alpha helix or beta strands). Such structures may be preferred due to incompatibility of a given amino acid side chain for a secondary structure formed by the amino acids preceding it. Bends occur commonly in proteins and proline is often implicated.

Bends do not have the predictable geometry of alpha helices or beta strands and are often referred to as random coils. Thus, even though protein structure can be described easily as regions of secondary structure separated by bends, the variability of bend structures makes prediction of tertiary

O Little Protein Molecule

To the tune of “*O Little Town of Bethlehem*”

Oh little protein molecule
You're lovely and serene
With twenty zwitterions like
Cysteine and alanine

Your secondary structure
Has pitches and repeats
Arranged in alpha helices
And beta pleated sheets

The Ramachandran plots are
Predictions made to try
To tell the structures you can have
For angles phi and psi

And tertiary structure
Gives polypeptides zing
Because of magic that occurs
In protein fol-ding

A folded enzyme’s active
And starts to catalyze
When activators bind into
The allosteric sites

Some other mechanisms
Control the enzyme rates
By regulating synthesis
And placement of phosphates

And all the regulation
That's found inside of cells
Reminds the students learning it
Of pathways straight from hell

Recorded by Tim Karplus
Lyrics by Kevin Ahern

structure from amino acid sequence enormously more difficult than identifying/ predicting regions of secondary structure.

A hydrophobic effect causes the water on this leaf to assume a spherical shape

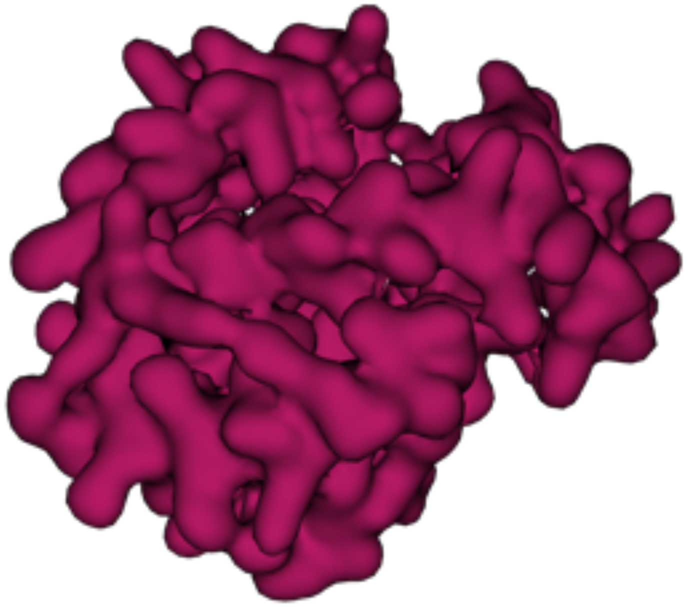
from Wikipedia

Hydrophobic Effect

It is at the level of tertiary structure that the characteristic arrangement of hydrophobic and hydrophilic amino acids in a protein occurs. In an aqueous environment, for a protein to remain soluble, it must have favorable interactions with the water around it, hence, the positioning of hydrophilic amino acids externally. Another impetus for the folding phenomenon is a bit harder to understand. It is known as the **hydrophobic effect**. At a chemical level, it makes sense – hydrophobic amino acids will ‘prefer’ to interact with each other internally and away from water. The driving force for this phenomenon, though, is a bit more conceptually difficult. Consider a bottle containing oil and water. As everyone knows, the two liquids will not mix and instead will form separate layers. A reasonable question might be why they do this instead of one existing as tiny globules inside of the other. The answer to that question, as well as the positioning of hydrophobic amino acids in the interior of water soluble proteins, is the hydrophobic effect.

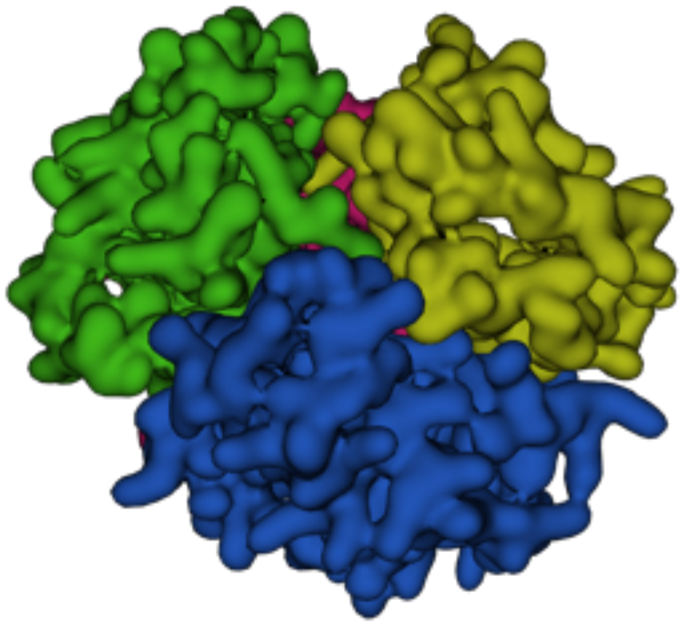
To understand the hydrophobic effect, perform the following experiment – take the water-oil mixture and shake it vigorously. This will force the layers to mix and one will observe that tiny globules of both water and oil can, in fact, be found initially in the layer of each. Over time, though, the tiny globules break up and merge with the appropriate layer. This is due to the phenomenon of entropy and consideration of surface area. First, the sum of the surface area of the embedded tiny globs is far greater than

Interactive 3.1



Oxygenated Myoglobin

Interactive 3.2

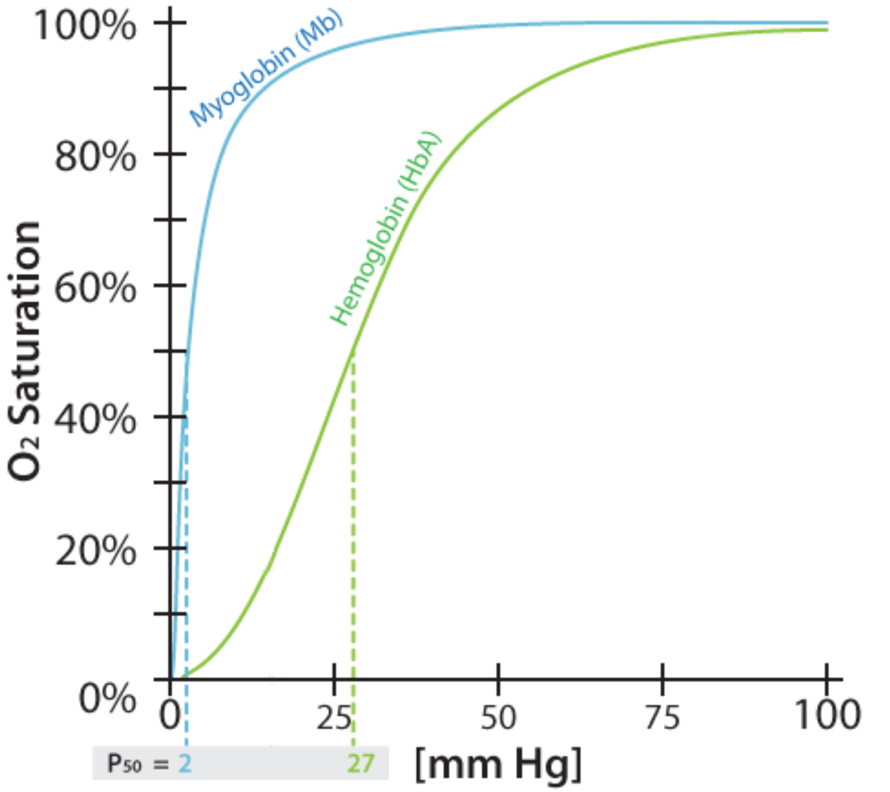


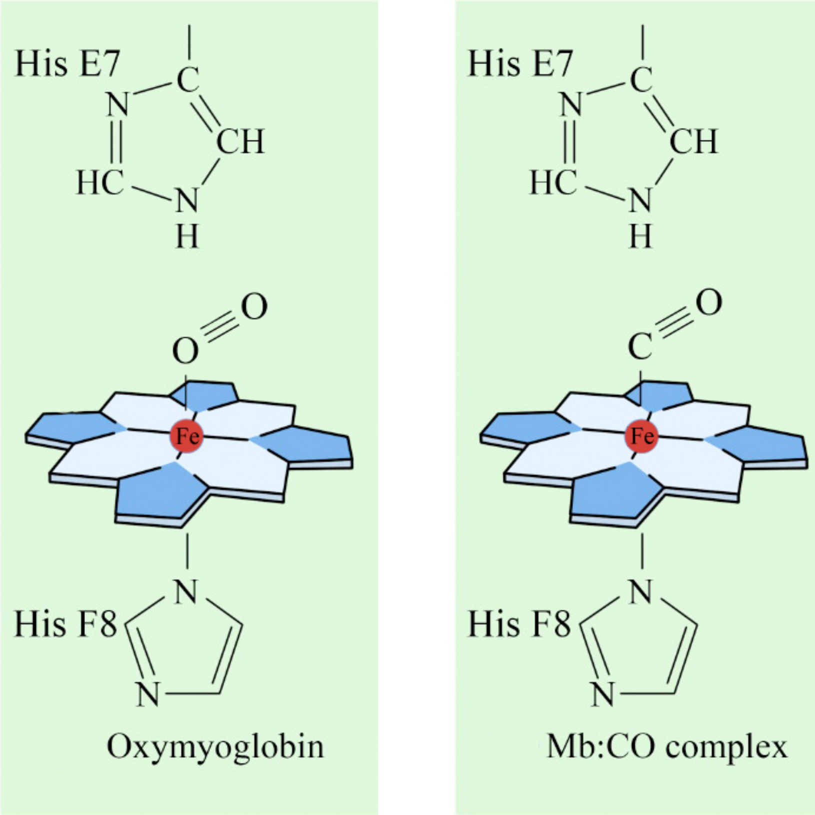
Hemoglobin in the Absence of Oxygen

the area of the region between the two layers after mixing is over. The smaller the globs, the more the surface area of interaction between the oil and the water. The minimum possible surface area of interaction occurs when there are no globs at all – just two layers and nothing else.

How does this relate to entropy? Interactions between the water-hydrophobic layers causes the molecules at the interface to arrange themselves precisely/regularly so as to minimize their interactions. Ordering thus occurs at the layer interfaces. The maximum amount of ordering occurs when the maximum surface areas of oil and water interact. Small globules give rise to more exposed surface area between the water and hydrophobic layers and, as a consequence, more ordering. Since **entropy** in a closed system tends to increase, it will tend to reduce the amount of ordering, if left alone. Thus, one can increase the ordering on a nanoscopic scale (forming globules) by applying energy in the form of shaking. When left alone, however, the system will increase its disorder by reducing the interactions between hydrophobic groups and hydrophilic ones.

In the oil water mixture, this causes the tiny globs to break up and produce the two layers we are familiar with because this is the minimum surface area that can be made between the two layers and thus the least ordering. In proteins, hydrophobic amino acid side chains are 'shielded' from water by placement internal to the protein, thus also reducing interfaces between hydrophobic residues and water. In both cases, entropy




His E7
C
N
CH
HC
N
H
O
O
Fe
His F8
N
N
Oxymyoglobin
His E7
C
N
CH
HC
N
H
C
O
Fe
His F8
N
N
Mb:CO complex

O_2 and CO Binding to Heme

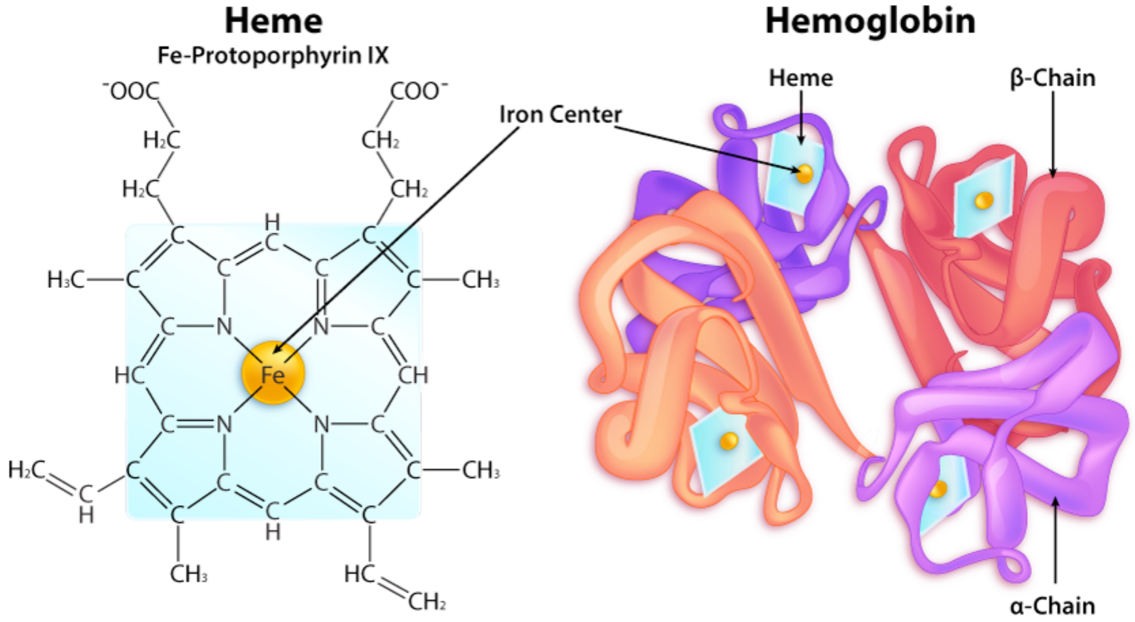
is increased, due to the reduced organization of the layers. Once formed, the interactions between the hydrophobic amino acid side chains helps to stabilize the overall protein structure.

Quaternary Structure

The last level of protein structure we will consider is that of **quaternary structure**. In order to have quaternary structure, a protein must have multiple polypeptide subunits because the structure involves the arrangement of those subunits with respect to each other. Consider **hemoglobin**, the oxygen-carrying protein of our blood. It contains two identical subunits known as alpha and two other identical ones known as beta. These are arranged together in a fashion as shown on the previous page. By contrast, the related oxygen storage protein known as myoglobin only contains a single subunit. Hemoglobin has quaternary structure, but myoglobin does not. Multiple subunit proteins are common in cells and they give rise to very useful properties not found in single subunit proteins. In the case of hemoglobin, the multiple subunits confer

Click HERE and HERE for Kevin's Hemoglobin lectures on YouTube

My blood has a proclivity
For co-op-ER-a-TIV-it-y
It's 'cause when in the lung environs
Ox-y-GEN binds to the irons
And changes hemoglobin's fate
Out of a T to the R-State


Heme
Fe-Protoporphyrin IX
⁻OOC
COO⁻
H₂C
CH₂
H₃C
CH₃
Fe
N
H
HC
CH
H₂C
CH₃
HC
CH₂
Iron Center
Hemoglobin
Heme
β-Chain
α-Chain

the property of cooperativity – variable affinity for oxygen depending on the latter's concentration. In the case of enzymes, it can impart **allosterism** – the ability to have the activity of the enzyme altered by interaction with an effector molecule. We will discuss allosterism in detail in the next chapter.

Other Protein Structural Features

Not everything found in a protein is an amino acid. Proteins frequently have other chemical groups, known as prosthetic groups, bound to them, that are necessary for the function of a protein. Examples include the **porphyrin** ring of heme in myoglobin and hemoglobin that carries an iron so that oxygen can be bound. Metals are frequently employed by enzymes in their catalysis. Several **vitamins** (referred to as **coenzymes**), such as **thiamine** (B_1) and **riboflavin** (B_2) are modified and chemically bound to enzymes to help them perform specific catalytic functions.

Cooperativity

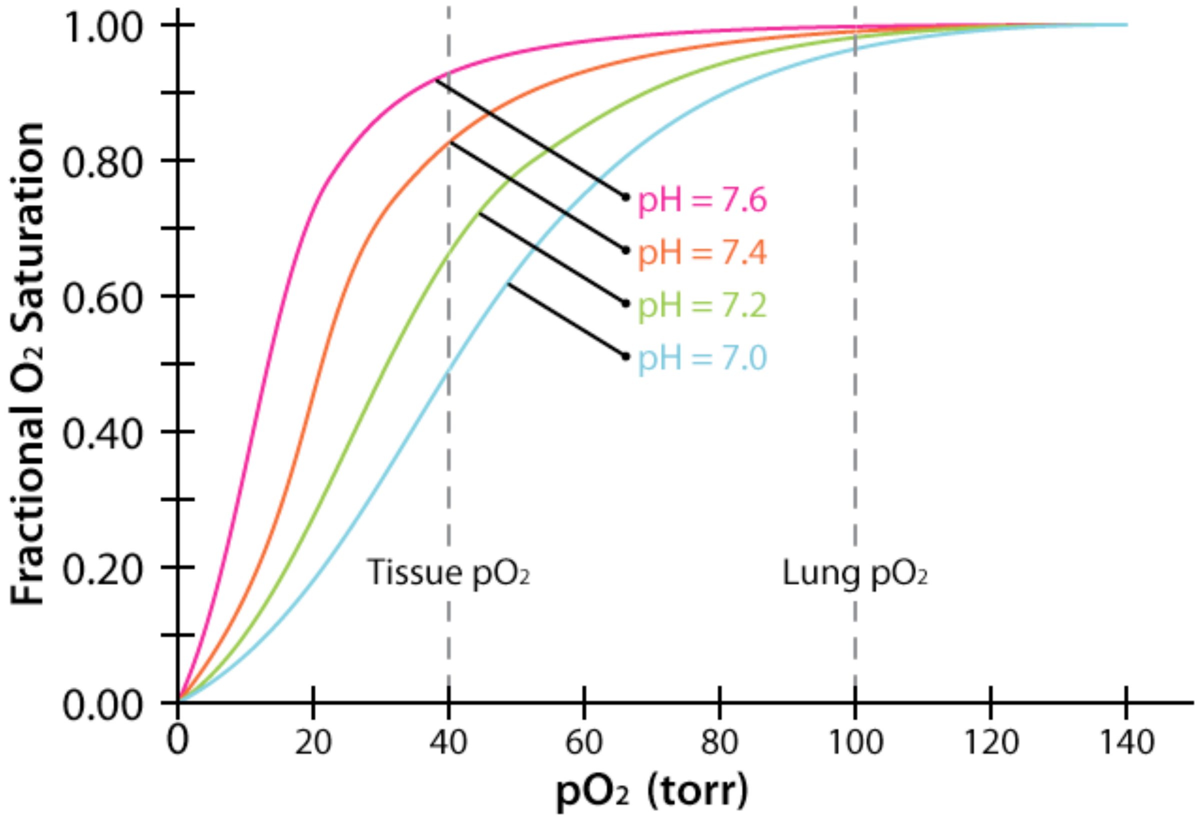
An interesting and important aspect of some proteins is the phenomenon of **cooperativity**. Cooperativity refers to the fact that binding of one ligand molecule by a protein favors the binding of additional molecules of the same type. Hemoglobin, for example, exhibits cooperativity when the binding of an oxygen molecule by the iron of the heme group in one of the four subunits causes a slight conformation change in the subunit. This happens because the heme iron is attached to a histidine side chain and binding of oxygen 'lifts' the iron along with the histidine ring (also known as the imidazole ring).

Since each hemoglobin subunit interacts with and influences the other subunits, they too are induced to change shape slightly when the first subunit binds to oxygen (a transition described as going from the T-state to the R-state). These shape changes favor each of the remaining subunits binding oxygen, as well. This is very important in the lungs where oxygen is picked up by hemoglobin, because the binding of the first oxygen molecule facilitates the rapid uptake of more oxygen molecules. In the tissues, where the oxygen concentration is lower, the oxygen leaves hemoglobin and the proteins flips from the R-state back to the T-state.

Cooperativity is only one of many fascinating structural aspects of hemoglobin that help the body to receive oxygen where it is needed and pick it up where it is abundant. Hemoglobin also assists in the transport of the product of cellular respiration (carbon dioxide) from the tissues producing it to the lungs where it is exhaled. Let us consider these individually.

Bohr Effect

The Bohr Effect was first described over 100 years ago by Christian Bohr. Shown graphically (above left), the observed effect is that hemoglobin's affinity for oxygen decreases as the pH

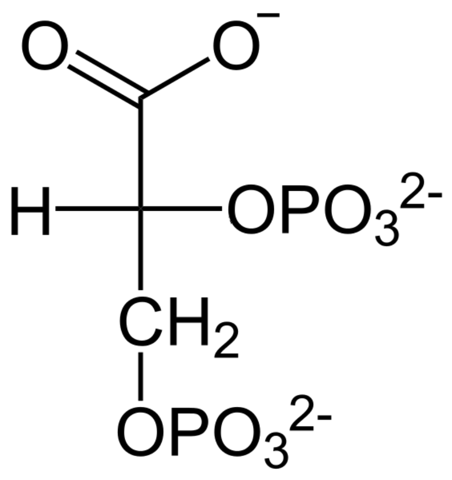


The Bohr Effect

decreases and/or as the concentration of carbon dioxide increases. Binding of the protons by histidine helps to facilitate structural changes in the protein and also with the uptake of carbon dioxide. Physiologically, this has great significance because actively respiring tissues (such as contracting muscles) require oxygen and release protons and carbon dioxide. The higher the concentration of protons and carbon dioxide, the more oxygen is released to feed the tissues that need it most.

2,3 BPG

Another molecule affecting the release of oxygen by hemoglobin is 2,3 bisphosphoglycerate (also called 2,3 BPG or just BPG). Like protons and carbon dioxide, 2,3 BPG is produced by actively respiring tissues, as a byproduct of glucose metabolism. The 2,3 BPG molecule fits into the 'hole of the donut' of adult hemoglobin. Such binding of 2,3 BPG favors the T (tight) state of hemoglobin, which has a reduced affinity for oxygen. In the absence of 2,3 BPG, hemoglobin can exist in the R (relaxed) state, which has a high affinity for oxygen.



2,3 BPG

Fetal Hemoglobin

Adult hemoglobin releases oxygen when it binds 2,3 BPG. This is in contrast to fetal hemoglobin, which has a slightly different configuration ($\alpha_2\gamma_2$) than adult hemoglobin ($\alpha_2\beta_2$). Fetal hemoglobin has a greater affinity for oxygen than maternal hemoglobin, allowing the fetus to obtain oxygen effectively from the mother's blood. Part of the reason for fetal hemoglobin's greater affinity for oxygen is that it doesn't bind 2,3 BPG.

Another significant fact about 2,3 BPG is that its concentration is higher in the blood of smokers than it is of non-smokers.

Hemoglobin's Moving Around

To the tune of "*Santa Claus is Coming to Town*"

Oh isn't it great
What proteins can do
Especially ones that bind to O2
Hemoglobin's moving around

Inside of the lungs
It picks up the bait
And changes itself from T to R state
Hemoglobin's moving around

The proto-porphyrin system
Its iron makes such a scene
Arising when an O2 binds
Pulling up on histidine

The binding occurs
Cooperatively
Thanks to changes qua-ter-nar-y
Hemoglobin's moving around

It exits the lungs
Engorged with O2
In search of a working body tissue
Hemoglobin's moving around

The proton concentration
Is high and has a role
Between the alpha betas
It finds imidazole

To empty their loads
The globins decree
"We need to bind 2,3BPG"
Hemoglobin's moving around

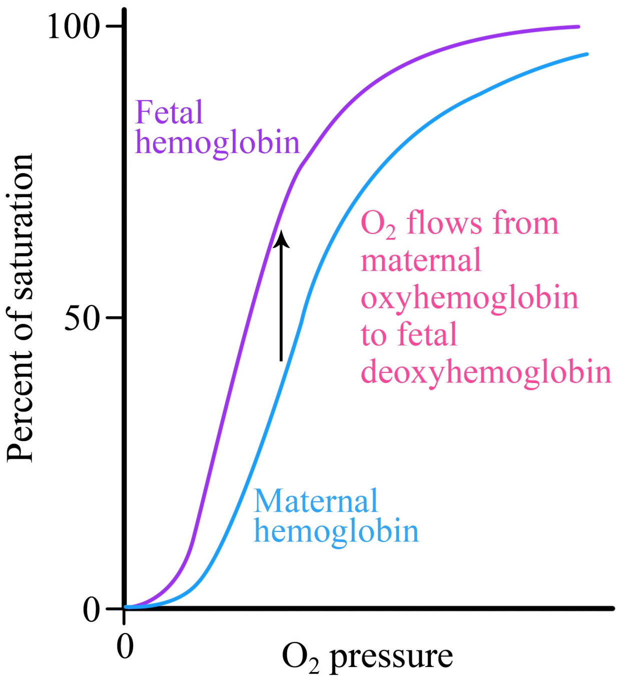
The stage is thus set
For grabbing a few
Cellular dumps of CO2
Hemoglobin's moving around

And then inside the lungs it
Discovers ox-y-gen
And dumps the CO2 off
To start all o'er agin

So see how this works
You better expect
To have to describe the Bohr effect
Hemoglobin's moving around

Recorded by Tim Karplus
Lyrics by Kevin Ahern

Consequently, hemoglobin in a smoker's blood spends more time in the T state than the R state. That is a problem when it is in the lungs, where being in the R state is necessary to maximally load the hemoglobin with oxygen. A high blood level of 2,3 BPG is one of the reasons smokers have trouble breathing when they exercise – they have reduced oxygen carrying capacity.



Fetal Hemoglobin Binding of O_2

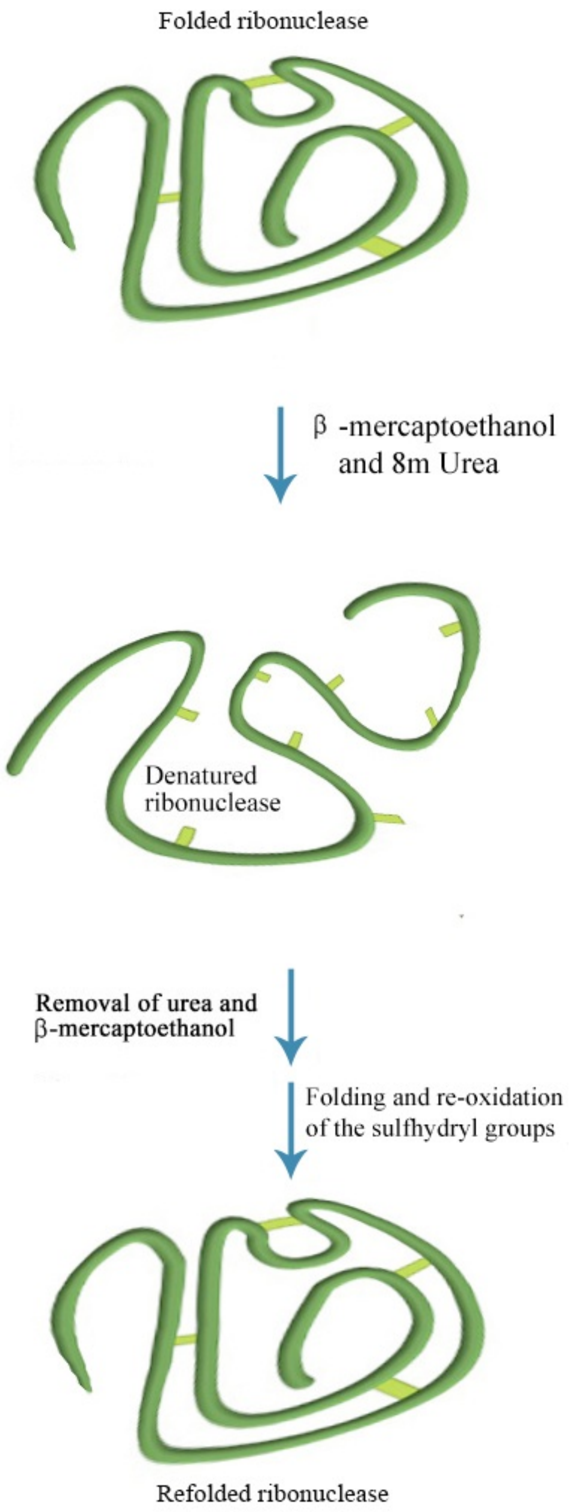
Last, though it is not related directly to 2,3 BPG, smokers have another reason why their oxygen carrying capacity is lower than that of non-smokers. Cigarette smoke contains carbon monoxide and this molecule, which has almost identical dimensions to molecular oxygen, competes effectively with oxygen for binding to the iron atom of heme. Part of carbon monoxide's toxicity is due to its ability to bind hemoglobin and prevent oxygen from binding.

Denaturation

For proteins, function is dependent on precise structure. Loss of the precise, folded structure of a protein is known as denaturation and is usually accompanied by loss of function. Anyone who has ever worked to purify an enzyme knows how easy it is for one to lose its activity. A few enzymes, such as ribonuclease, are remarkably stable under even very harsh conditions. For most others, a small temperature or pH change can drastically affect activity. The reasons for these differences vary, but relate to 1) the strength of the forces holding the structure together and 2) the ability of a protein to refold itself after being denatured. Let us consider these separately below.

Forces Stabilizing Structure

Amino acids are linked one to the other by peptide bonds. These covalent bonds are extraordinarily stable at neutral pHs, but can be broken by hydrolysis with heat under acidic conditions. Peptide bonds, however, only stabilize primary structure and, in fact, are the only relevant force responsible for it. Secondary structure, on the other hand, is generally stabilized by weaker forces, including hydrogen bonds. Hydrogen bonds are readily disrupted by heat, urea, or guanidinium chloride.


Folded ribonuclease
β -mercaptoethanol and 8m Urea
Denatured ribonuclease
Removal of urea and β-mercaptoethanol
Folding and re-oxidation of the sulfhydryl groups
Refolded ribonuclease

Denaturation and Renaturation of Ribonuclease

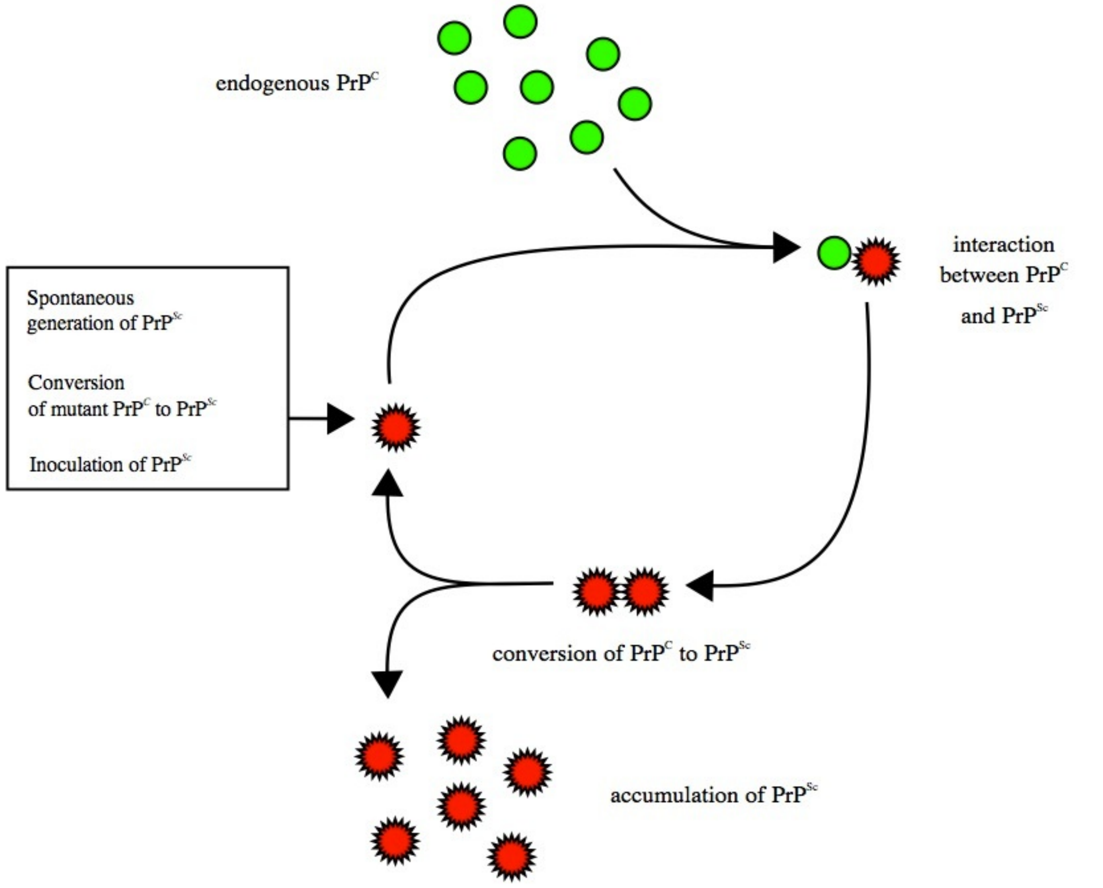
Forces stabilizing tertiary structure include ionic interactions, disulfide bonds, hydrophobic interactions, metallic bonds, and hydrogen bonds. Of these, the ionic interactions are most sensitive to pH changes. Hydrophobic bonds are most sensitive to detergents. Thus, washing one's hands helps to kill bacteria by denaturing critical proteins they need to survive. Metallic bonds are sensitive to oxidation/reduction. Breaking disulfide bonds requires either a strong oxidizing agent, such as performic acid or a strong reducing agent on another disulfide, such as mercaptoethanol or dithiothreitol.

Quaternary structures are stabilized by the same forces as tertiary structure and have the same sensitivities.

Refolding Denatured Proteins

All of the information for protein folding is contained in the primary structure of the protein. It may seem curious then that most proteins do not refold into their proper, fully active form after they have been denatured and the denaturant is removed. A few do, in fact, refold correctly under these circumstances. A good example is bovine ribonuclease (also called RNase). Its catalytic activity is very resistant to heat and urea. However, if one treats the enzyme with mercaptoethanol (which breaks disulfide bonds) prior to urea treatment and heating, activity is lost, indicating that the covalent disulfide bonds help stabilize the overall enzyme structure. If one allows the enzyme mixture to cool back down to room temperature, over time some enzyme activity reappears, indicating that ribonuclease can re-fold under the proper conditions.

There are not very many ways
Inactivating RNase
It's stable when it's hot or cold
Because disulfides tightly hold
If you desire to make it stall
Use hot mercaptoethanol


endogenous PrP^{C}
interaction between PrP^{C} and PrP^{Sc}
Spontaneous generation of PrP^{Sc}
Conversion of mutant PrP^{C} to PrP^{Sc}
Inoculation of PrP^{Sc}
conversion of PrP^{C} to PrP^{Sc}
accumulation of PrP^{Sc}

from Wikipedia

Prion Protein Misfolding

Irreversible Denaturation

Most enzymes, however, do not behave like ribonuclease. Once denatured, their activity cannot be recovered to any significant extent. This may seem to contradict the idea that folding information is inherent to the sequence of amino acids in the protein. It does not. The reason most enzymes can't refold properly is due to two phenomena. First, normal folding may occur as proteins are being made. Interactions among amino acids early in the synthesis are not "confused" by interactions with amino acids later in the synthesis because those amino acids aren't present as protein synthesis starts. In many cases, the proper folding of newly made polypeptides is also assisted by special proteins called chaperones. Chaperones bind to newly made proteins, preventing interactions that might result in misfolding. Thus, early folding and the assistance of chaperones eliminate some potential “wrong-folding” interactions that can occur if the entire sequence was present when folding started.

Denatured full-length polypeptides have many more potential wrong folds that can occur. A second reason most proteins don’t refold properly after denaturation is probably that folding, like any other natural phenomenon, is driven by energy minimization. Though the folded structure may have a low energy, the path leading to it may not be all downhill. Like a chemical reaction that has energies of activation that must be overcome for the reaction to occur, folding likely has peaks and valleys of energy that do not automatically lead directly to the proper fold. Again, folding during synthesis leads the protein along a better-defined path through the energy maze of folding that denatured full-length proteins can’t navigate.

I think that if I chanced to be on
The protein making up a prion
I’d twist it and for goodness sakes
Stop it from making fold mistakes

Prions and Misfolding

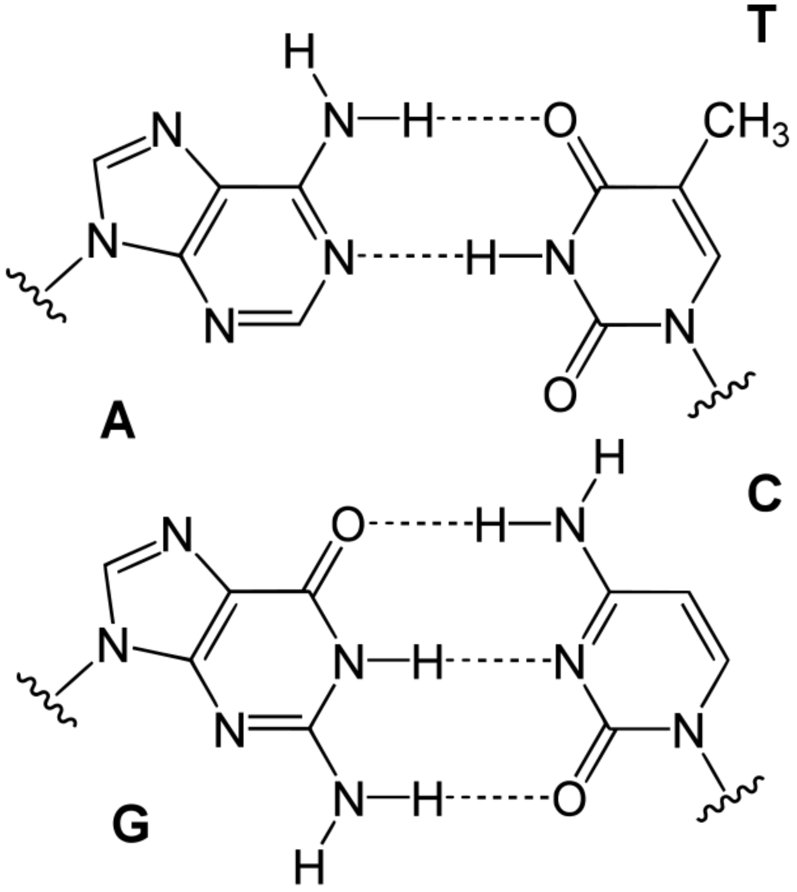
Folding and the stability of folded proteins is an important consideration for so-called “infectious” proteins known as prions. These mysterious proteins, which are implicated in diseases, such as mad cow disease and the related human condition known as Creutzfeldt-Jakob disease, result from the improper folding of a brain protein known as PrP. The misfolded protein has two important properties that lead to the disease. First, it tends to aggregrate into large complexes called amyloid plaques that damage/destroy nerve cells in the brain, leading ultimately to dementia and loss of brain function.

Second, and probably worse, the misfolded protein “induces” other copies of the same protein to misfold as well. Thus, a misfolded protein acts something like a catalytic center and the disease progresses rapidly. The question arises as to how the PrP protein misfolds to begin with, but the answer to this is not clear. There are suggestions that exposure in the diet to misfolded proteins may be a factor, but this is disputed. An outbreak of mad cow disease in Britain in the 1980s was followed by a rise in the incidence of a rare form of human Creutzfeld-Jakob disease called variant CJD (v-CJD), lending some credence to the hypothesis. It is possible that misfolding of many proteins occurs sporadically without consequence or observation, but if PrP misfolds, the results are readily apparent. Thus, Creutzfeld-Jakob disease may ultimately give insights into the folding process itself.

Nucleic Acids

Determination of the structure of the most common form of DNA, known as the B form, was one of the most important scientific advances of the 20th century. Using data from **Rosalind Franklin**, **James Watson** and

Click HERE for Kevin’s YouTube lecture on Nucleic Acid Structure



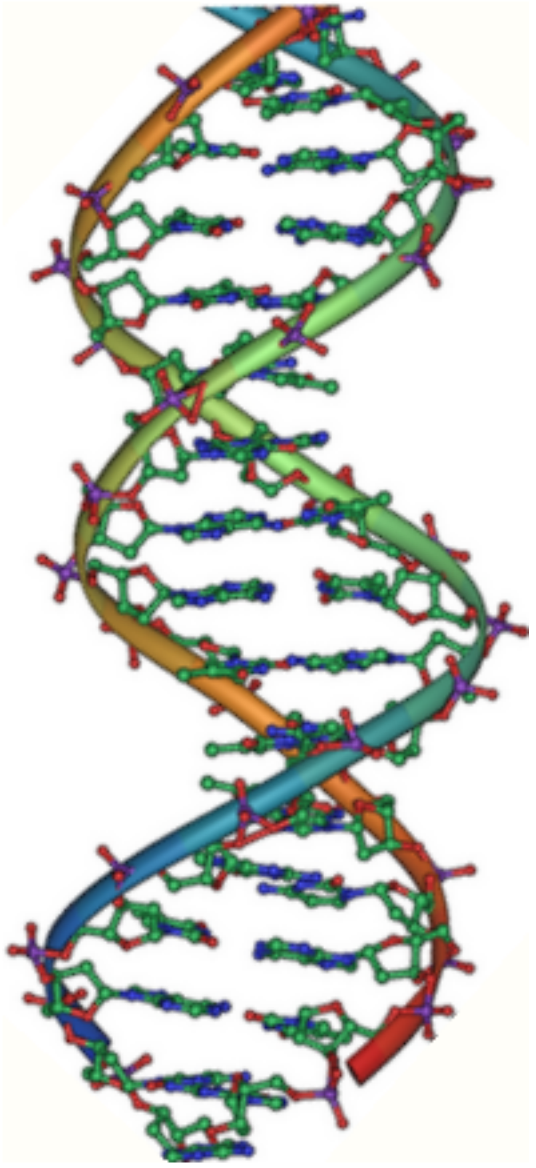
A-T Base Pairs (Top) and G-C Base Pairs (Bottom)

Francis Crick initiated the modern era of molecular biology with their paper in the April 25, 1953 issue of Nature. Arguably, that single page paper has had more scientific impact per word than any other research article ever published. Today, every high school biology student knows the double helical structure in which G pairs with C and A pairs with T. The DNA molecule is a polymer of nucleoside monophosphates with **phosphodiester bonds** between the phosphate and the 5' end of one deoxyribose and the 3' end of the next one. In the B form the DNA helix has a repeat of 10.5 base pairs per turn, with sugars and phosphate forming the covalent "backbone" of the molecule and the adenine, guanine, cytosine, and thymine bases oriented in the middle where they form the now familiar base-pairs that look like the rungs of a ladder.

Hydrogen bonds help to hold the base pairs together, with two hydrogen bonds per A-T pair and three hydrogen bonds per G-C pair. The two strands of a DNA duplex run in opposite directions. The 5' end of one strand is paired with the 3' end of the other strand and vice-versa at the other end of the duplex. The B form of DNA has a prominent major groove and a minor groove tracing the path of the helix (shown at left). Proteins, such as transcription factors bind in these grooves and access the hydrogen bonds of the base pairs to "read" the sequence therein.

Other forms of DNA besides the B form are known. One of these, the 'A' form, was identified by Rosalind

DNA



DNA Double Helix

Major Groovy

To the tune of "*Feelin' Groovy*"

The DNA forms
A and B
Have bases
Complementary
Despite the similarities
They differ in their
Major groovies
Nanananananana major groovies

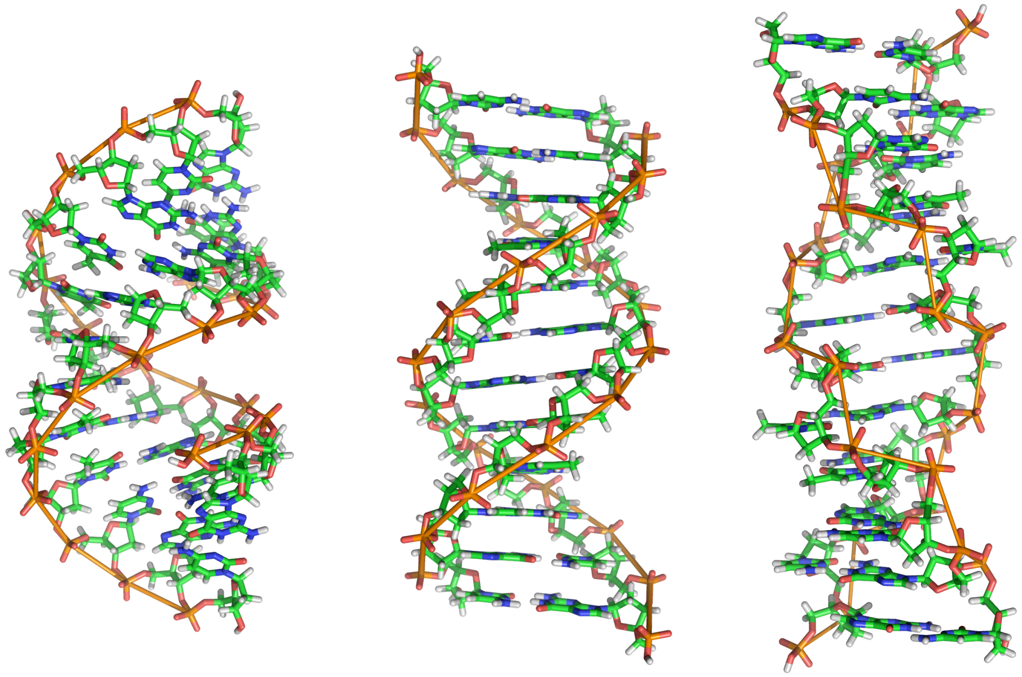
Transcription factors
With their bindin'
Cause DNA to
Start unwindin'
Holding it
Aggressively
By forming bonds
In major groovies
Nanananananana major groovies

For proteins, the key
To sequence I-D
Is hydrogen bonding, each base pair unique
Purine, pyrimidine patterns discrete
In DNA's most
Major groovy
Nanananananana major groovy

Recorded by David Simmons
Lyrics by Kevin Ahern

Franklin in the same issue of Nature as Watson and Crick's paper. Though the A structure is a relatively minor form of DNA and resembles the B form, it turns out to be important in the duplex form of RNA and in RNA-DNA hybrids. Both the A form and the B form of DNA have the helix oriented in what is termed the right-handed form.

These stand in contrast to another form of DNA, known as the Z form. Z-DNA, as it is known, has the same base-pairing rules as the B and A forms, but instead has the helices twisted in the opposite direction, making a left-handed helix (see figure on previous page). The Z form has a sort of zig-zag shape, giving

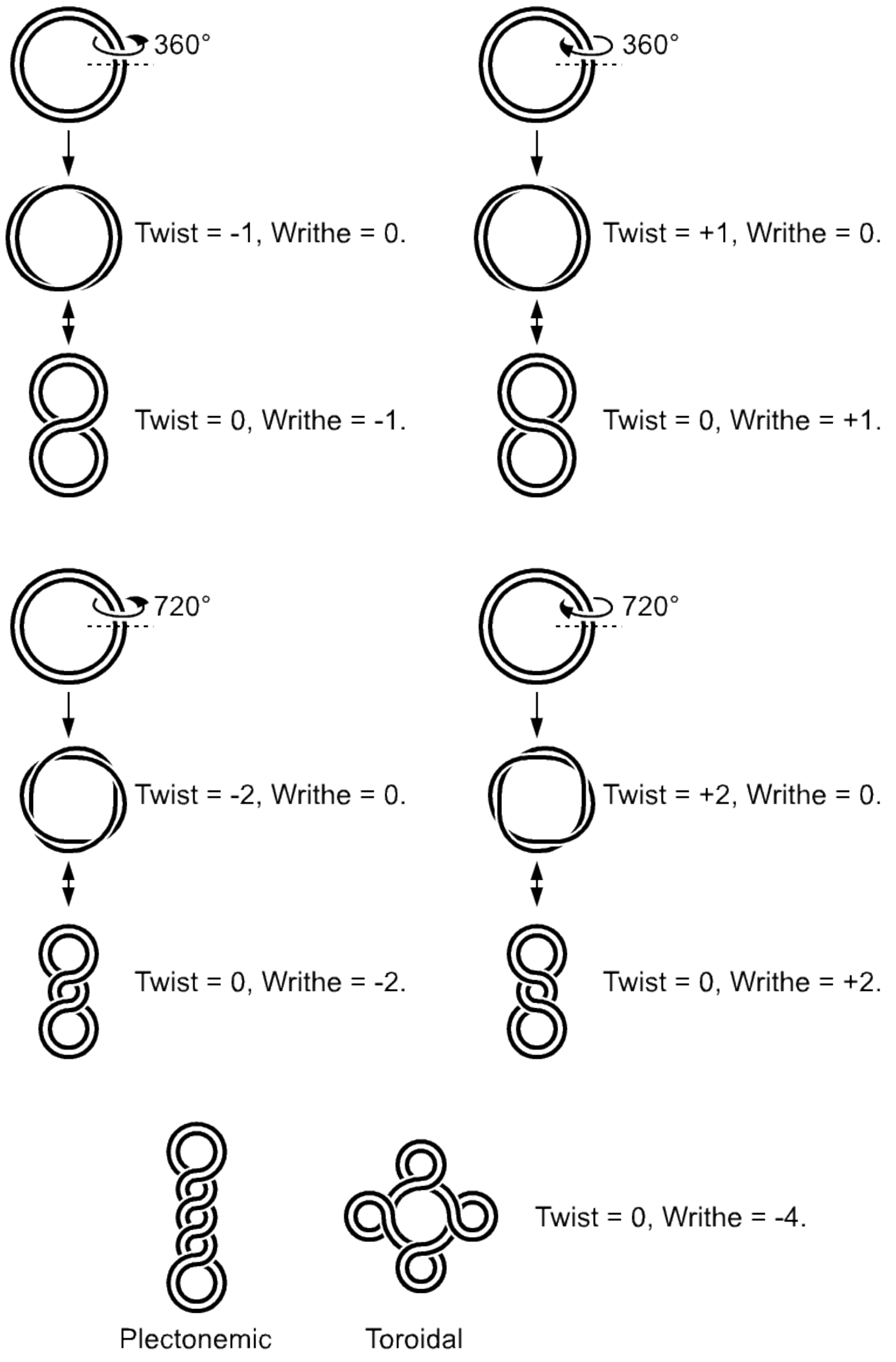


A, B, and Z Forms of DNA

from Wikipedia

It’s taught in school of DNA
The race for structure underway
Gave rise to competition huge
Along with data subterfuge
In view of this we should extend
New authorship to make amends
A fairer order could be picked
Franklin, Wilkins, Watson, Crick

rise to the name Z DNA. In addition, the helix is rather stretched out compared to the A and B forms. Why are there different forms of DNA? The answer relates to both superhelical tension and sequence bias. Sequence bias means that certain sequences tend to favor the “flipping” of B form DNA into other forms. Z DNA forms are favored by long stretches of alternating Gs and Cs. Superhelical tension will be discussed below.


360°
360°
Twist = -1, Writhe = 0.
Twist = +1, Writhe = 0.
Twist = 0, Writhe = -1.
Twist = 0, Writhe = +1.
720°
720°
Twist = -2, Writhe = 0.
Twist = +2, Writhe = 0.
Twist = 0, Writhe = -2.
Twist = 0, Writhe = +2.
Twist = 0, Writhe = -4.
Plectonemic
Toroidal

Topoisomers of DNA

from Wikipedia

Superhelicity

Short stretches of linear DNA duplexes exist in the B form and have 10.5 base pairs per turn. Double helices of DNA in the cell can vary in the number of base pairs per turn they contain. There are several reasons for this. For example, during DNA replication, strands of DNA at the site of replication get unwound at the rate of 6000 rpm by an enzyme called helicase. The effect of such local unwinding at one place in a DNA has the effect increasing the winding ahead of it. Unrelieved, such ‘tension’ in a DNA duplex can result in structural obstacles to replication.

Such adjustments can

B-DNA

To the tune of "*Y-M-C-A*"

Phosphates
Are in nucleotides
I say phosphates
Cover bases inside
I say phosphates
Span the 5 and 3 primes
There's no need - to - be - real - mixed - up

Bases
Carry info you see
I say bases
Are all complement'ry
I say bases
Like A,T,G and C
They have got - to - be – all - paired - up

It's fun to play with some B-DNA
It's got a boatload of G-C-T-A
It's got everything
A polymerase needs
When you melt all the A's and T's

It's fun to play with some B-DNA
It's got a boatload of G-C-T-A
You can make RNAs
With a po-ly-mer-ase
Just by pairing up U's with A's

Proteins
Full of amino A's
I say proteins
Come from mRNAs
I say proteins
Require tRNAs
There is more – you - need - to – trans-late

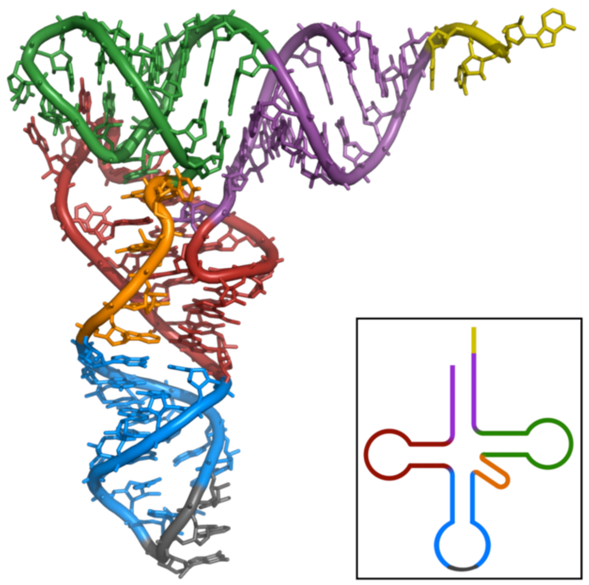
Codons
Like our friend U-A-C
I say codons
Come in clusters of three
I say codons
Have one base wobble –ee
Now you can - go - forth - and - tran-slate

It's fun to play with some B-DNA
It's got a boatload of G-C-T-A
With those hydrogen Bs
And right-hand he-li-ces
Anti-par-a-llel fives and threes

It's fun to play with some B-DNA
It's got a boatload of G-C-T-A
With those hydrogen Bs
And right-hand he-li-ces
Anti-par-a-llel fives and threes

B-DNA

Recorded by Tim Karplus
Lyrics by Kevin Ahern



3D tRNA Structure (left) and 2D projection (right)

from Wikipedia

occur in three ways. First, tension can provide the energy for ‘flipping’ DNA structure. Z-DNA can arise as a means of relieving the tension. Second, DNA can ‘supercoil’ to relieve the tension. In this method, the strands of the duplex can cross each other repeatedly, much like a rubber band will coil up if one holds one section in place and twists another part of it. Third, enzymes called **topoisomerases** can act to relieve or, in some cases, increase the tension by adding or removing twists in the DNA.

RNA Structures

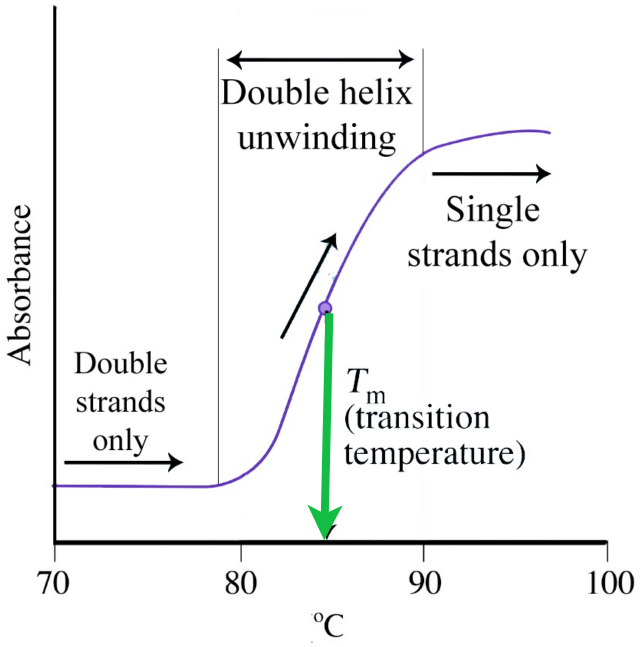
With respect to structure, RNAs are more varied than their DNA cousins. Created by copying regions of DNA, cellular RNAs are synthesized as single strands, but they often have self-complementary regions leading to “fold-backs” containing duplex regions. The structure of **tRNAs** and **rRNAs** are excellent examples. The base-pairing rules of DNA are the same in RNA (with U in RNA replacing the T from DNA), but in addition, base pairing between G and U can also occur in RNA. This latter fact leads to many more possible duplex regions in RNA that can exist compared to single strands of DNA.

RNA structure, like protein structure, has importance, in some cases, for catalytic function. Like random coils in proteins that give rise to tertiary structure, single-stranded regions of RNA that link duplex regions give these molecules a tertiary structure, as well. Catalytic RNAs, called ribozymes, catalyze important cellular reactions, including the formation of peptide bonds. DNA, which is usually present in cells in strictly duplex forms (no tertiary structure, per se), is not known to be involved in catalysis.

RNA structures are important for reasons other than catalysis. The 3D arrangement of tRNAs is important for enzymes that attach amino acids to them to do so properly. Small RNAs called siRNAs found in the nucleus of cells appear to play roles in both gene regulation and in cellular defenses against viruses. The key to the mechanisms of these actions is the formation of short fold-back RNA structures that are recognized by cellular proteins and then chopped into smaller units. One strand is copied and used to base pair with specific mRNAs to prevent the synthesis of proteins from them.

Denaturing Nucleic Acids

Like proteins, nucleic acids can be denatured. Forces holding duplexes together include hydrogen bonds between the bases of each strand that, like the hydrogen bonds in proteins, can be broken with heat or urea. (Another important stabilizing force for DNA arises from the stacking interactions between the bases in a strand.) Single strands absorb light at 260 nm more strongly than double strands (hyperchromic effect), allowing one to easily follow denaturation. For DNA, strand separation and strand hybridization are important aspects of the technique known as the polymerase chain reaction (PCR). Strand separation of DNA duplexes is accomplished in the method by heating them to boiling. Hybridization is an important aspect of the method that requires complementary single strands to “find” each other and form a duplex. Thus, DNAs (and RNAs too) can renature readily, unlike most proteins. Considerations for efficient hybridization (also called annealing) include temperature, salt concentration, strand concentration, and magnesium ion levels.



Hyperchromic Effect

Carbohydrates

The last class of macromolecules we will consider structurally here is the carbohydrates. Built of sugars or modified sugars, carbohydrates have several important functions, including structural integrity, cellular identification, and energy storage.

Monosaccharides

Simple sugars, also known as monosaccharides, can generally be written in the form $C_x(H_2O)_x$. It is for this reason they are referred to as carbo-hydrates. By convention, the letters ‘ose’ at the end of a biochemical name flags a molecule as a sugar. Thus, there are glucose, galactose, sucrose, and many other ‘-oses’. Other descriptive nomenclature involves use of a prefix that tells how many carbons the sugar contains. For example, glucose, which contains six carbons, is described as a hexose. The following list shows the prefixes for numbers of carbons in a sugar:

- Tri- = 3
- Tetr- = 4
- Pent- = 5
- Hex- =6
- Hept- = 7
- Oct- = 8

Other prefixes identify whether the sugar contains an aldehyde group (aldo-) or a ketone (keto) group. Prefixes may be combined. Glucose, which contains an aldehyde group, can be described as an aldo-hexose. The list that follows gives some common sugars and some descriptors.

- Ribose = aldo-pentose
- Glucose = aldo-hexose
- Galactose = aldo-hexose
- Mannose = aldo-hexose
- Glyceraldehyde = aldo-triose
- Erythrose – aldo-tetrose
- Fructose = keto-hexose
- Ribulose = keto-pentose
- Sedoheptulose = keto-heptose
- Dihydroxyacetone = keto-triose

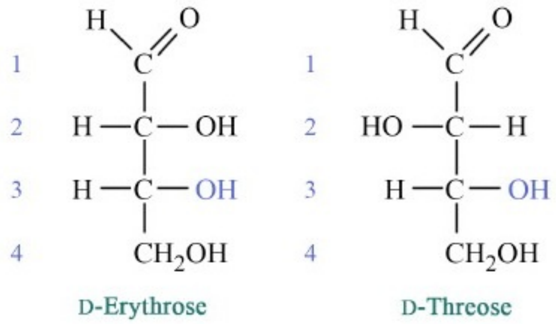
Stereoisomer Nomenclature

Sugars of a given category (hexoses, for example) differ from each other in the stereoisomeric configuration of their carbons. Two sugars having the same number of carbons (hexoses, for example) and the same chemical form (aldoses, for example), but differing in the stereoisomeric configuration of their carbons are called diastereomers. Biochemists use D and L nomenclature to describe sugars, as explained below.

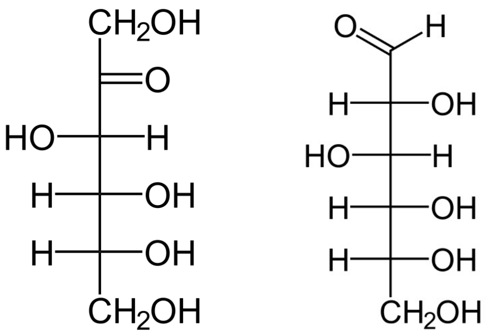
D-sugars predominate in nature, though L-forms of some sugars, such as fucose, do exist. The D and L designation is a bit more complicated than it would appear on the surface. To determine if a sugar is a D-sugar or an L-sugar, one simply examines the configuration of the highest numbered asymmetric carbon. If the hydroxyl is written to the right, it is a D-sugar. If the hydroxyl is on the left, it is an L-sugar. That part is simple. The confusion about D and L arises because L sugars of a given name (glucose, for example) are mirror images of D sugars of the same name. The figure on the previous page shows the structure of D- and L-glucose. Notice that D-glucose is not converted into L-glucose simply by flipping the configuration of the fifth carbon in the molecule. There is another name for sugars that are mirror images of each other. They are called **enantiomers**. Thus, L-glucose and D-glucose are enantiomers, but **D-Erythrose** and **D-Threose** are diastereomers.

Click HERE and HERE for Kevin's YouTube lectures on Carbohydrate Structure

Sugars of 5-7 carbons can fairly easily form ring structures (called **Haworth structures**). For aldoses like glucose, this involves formation of a hemi-acetal. For ketoses like fructose, it involves formation of a hemi-ketal. The bottom line for both is that the oxygen that was part of the aldehyde or the ketone group is the one that becomes a part of the ring. More important than the oxygen, though, is the fact that the carbon attached to it (carbon #1 in aldoses or #2 in



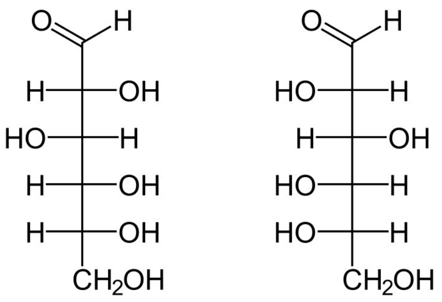
Diastereomers



D-Fructose D-Glucose

Ketose and Aldose

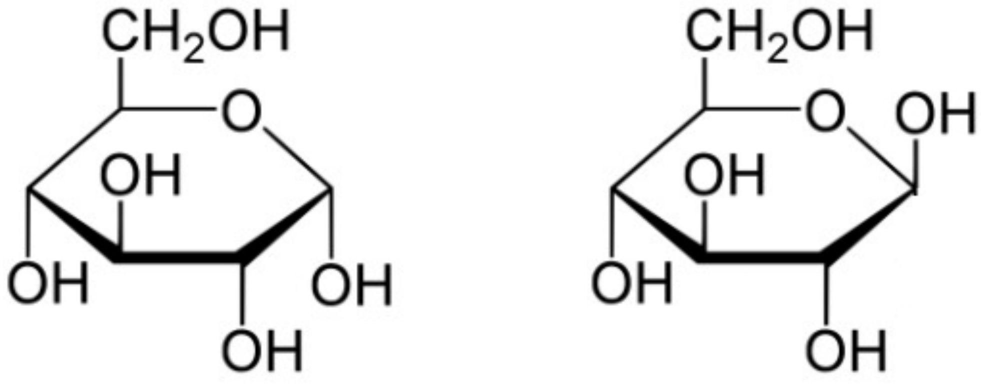
ketoses) becomes asymmetric as a byproduct of the cyclization. This new asymmetric carbon is called the **anomeric carbon** and it has two possible configurations, called alpha and beta.



D-Glucose L-Glucose

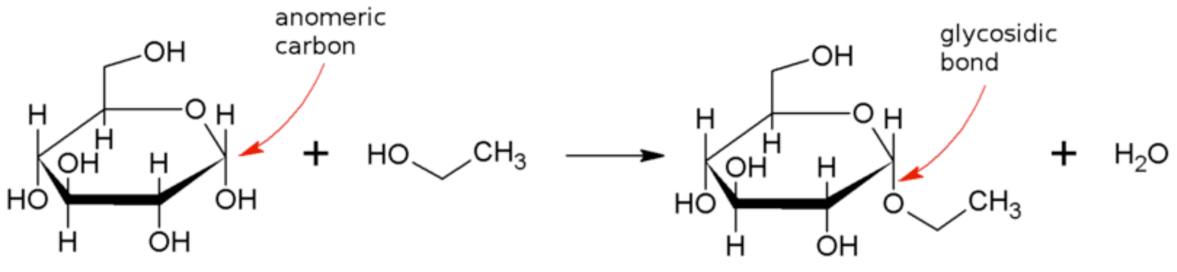
Enantiomers

A solution of glucose will contain a mixture of alpha and beta forms. Whether the alpha or the beta arises upon cyclization is partly determined by geometry and partly random. Thus, one can find a bias for one form, but usually not that form exclusively. A given molecule of sugar will flip between alpha and beta over time. A requirement for this is that the hydroxyl on the anomeric carbon is unaltered, thus facilitating flipping back to the straight chain form followed by recyclization. If the hydroxyl becomes chemically altered in any way (for example, replacement of its hydrogen by a methyl group), a **glycoside** is formed. Glycosides are locked in the same alpha or beta configuration they were in when the modification was made. Glycosides are commonly found in nature. **Sucrose**, for example , is a di-glycoside – both the glucose and the fructose have had their anomeric hydroxyls altered by being joined together.



α-D Glucose (left) and β-D Glucose (right)

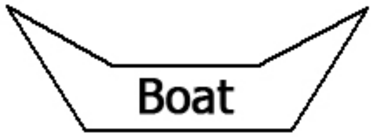
The last considerations for sugars relative to their structure are their chemical reactivity and modification. The aldehyde group of aldoses is susceptible to oxidation, whereas ketoses are less so. Sugars that are readily oxidized are called ‘**reducing sugars**’ because their oxidation causes other reacting molecules to be reduced. Reducing sugars can easily be identified in a chemical test. Chemical modification of sugars occurs readily in cells. As we will see, phosphorylation of sugars occurs routinely during metabolism. Oxidation of sugars to create carboxyl groups also can occur. Reduction of aldehyde/ketone groups of sugars creates what are called sugar alcohols, and other modifications, such as addition of sulfates and amines also readily occur.

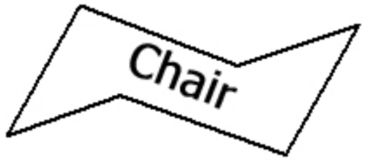


Glycoside Formation

Boat/Chair Conformations

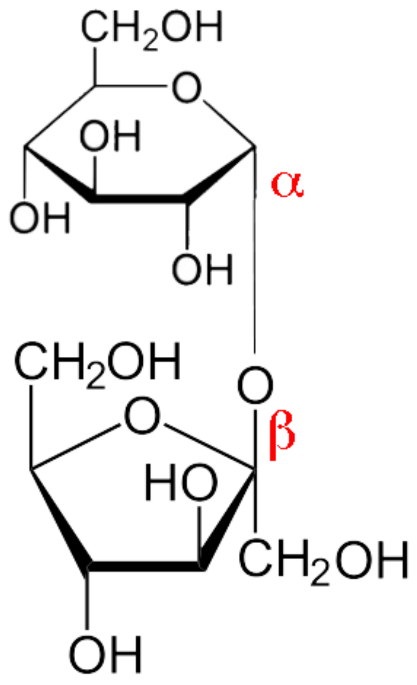
Independent of stereoisomerization, sugars in ring form of a given type (such as glucose) can “twist” themselves into alternative conformations called **boat** and **chair**. Note that this rearrangement does not change the relative positions of hydroxyl groups. All that has changed is the shape of the molecule. As shown for glucose, one can see that the beta-hydroxyl of glucose is closer to the CH_2OH (carbon #6) in the boat form than it is in the chair form. Steric hindrance can be a factor in favoring one configuration over another.





Disaccharides

Sugars are readily joined together (and broken apart) in cells. Sucrose (right), which is common table sugar, is made by joining the anomeric hydroxyl of alpha-D-glucose to the anomeric hydroxyl of beta-D-fructose. Not all **disaccharides** join the anomeric hydroxyls of both sugars. For example, **lactose** (milk sugar) is made by linking the anomeric hydroxyl of **galactose** in the beta configuration to the hydroxyl of carbon #4 of glucose.



Sucrose

Oligosaccharides

The term ‘**oligosaccharide**’ is used to describe polymers of sugars of 5-15 units, typically. Oligosaccharides are not commonly found free in cells, but instead are found covalently attached to proteins, which are then said to be glycosylated. Oligosaccharides attached to proteins may be N-linked (through asparagine) or O-linked (though serine or threonine). **O-linked sugars** are added only in the Golgi apparatus while **N-linked sugars** are attached starting in the endoplasmic reticulum and then completed in the Golgi.

Oligosaccharides often function as identity markers, both of cells and proteins. On the cell surface, glycoproteins with distinctive oligosaccharides attached establish the identity of each cell. The types of oligosaccharides found on the surface of blood cells is a determinant of blood type.

Hark the Sucrose

To the tune of "*Hark the Herald*"

Carbohydrates all should sing
Glory to the Haworth ring

Anomeric carbons hide
When they're in a glycoside

Glucopyranose is there
In the boat or in the chair

Alpha, beta, D and L
Di-astere-omer hell

Alpha, beta, D and L
Di-astere-omer hell

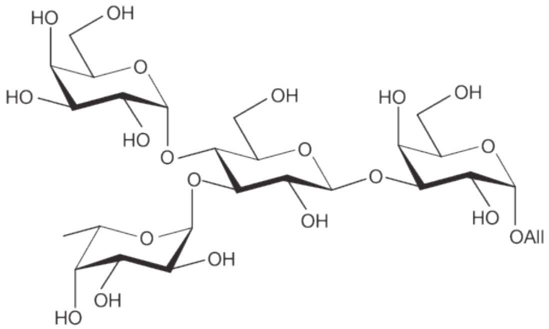
Recorded by Tim Karplus
Lyrics by Kevin Ahern

The oligosaccharides that are attached to proteins may also determine their cellular destinations. Improper glycosylation or sugar modification patterns can result in the failure of proteins to reach the correct cellular compartment. For example, inclusion cell (I-cell) disease arises from a defective phosphotransferase in the Golgi. This enzyme normally catalyzes the addition of a phosphate to a mannose sugar attached to a protein destined for the lysosome. In the absence of a functioning enzyme, the unphosphorylated glycoprotein never makes it to the lysosome and is instead exported out of the cell where it accumulates in the blood and is excreted in the urine. Individuals with I-cell disease suffer developmental delays, abnormal skeletal development, and restricted joint movement.

Click HERE and HERE for Kevin's YouTube lectures on Carbohydrate Structure

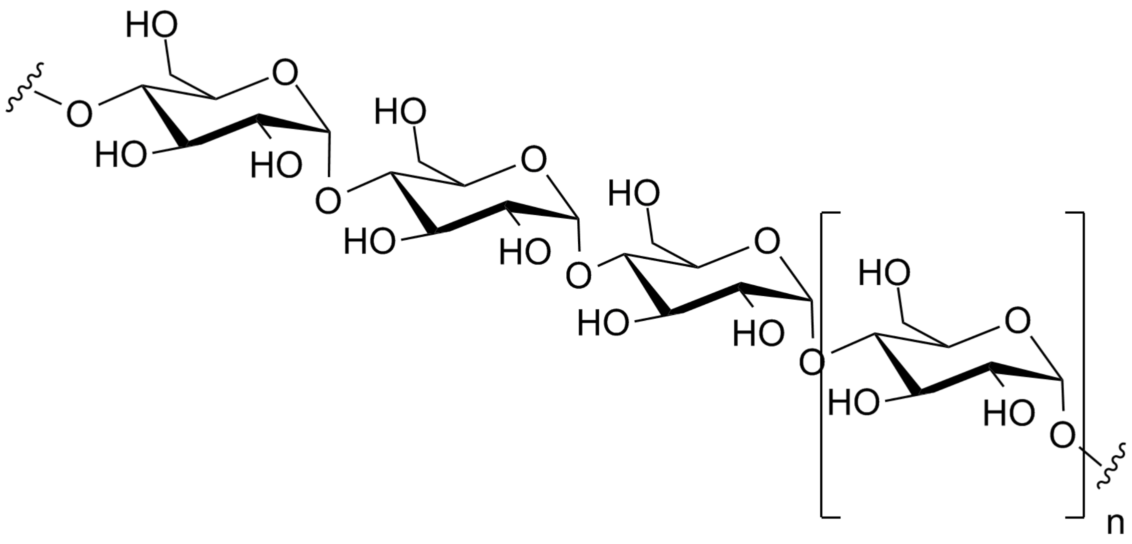
Polysaccharides

Polysaccharides, as their name implies, are made by joining together many sugars. The functions for polysaccharides are varied. They include energy storage, structural strength, and lubrication. Polysaccharides involved in energy storage include the plant polysaccharides, amylose and amylopectin. The polysaccharide involved in energy storage in animals is called glycogen and it is mostly found in the muscles and liver.



An oligosaccharide

from Wikipedia



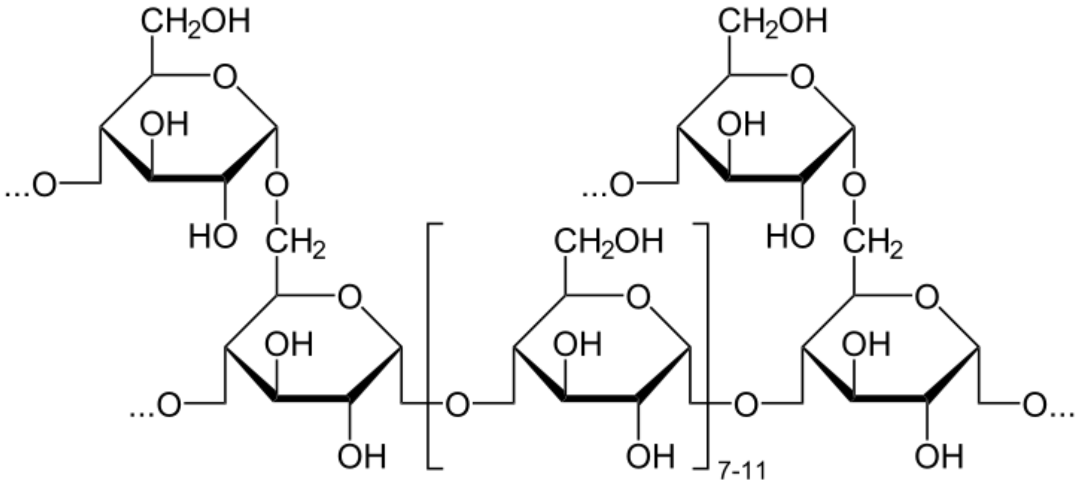
Amylose

Amylose/Amylopectin

Amylose is the simplest of the polysaccharides, being comprised solely of glucose units joined in an alpha 1-4 linkage. Amylose is broken down by the enzyme alpha-amylase, found in saliva. **Amylopectin** is related to amylose in being composed only of glucose, but it differs in how the glucose units are joined together. Alpha 1-4 linkages predominate, but every 30-50 residues, a ‘branch’ arises from an alpha 1-6 linkage. Branches make the structure of amylopectin more complex than that of amylose.

Glycogen

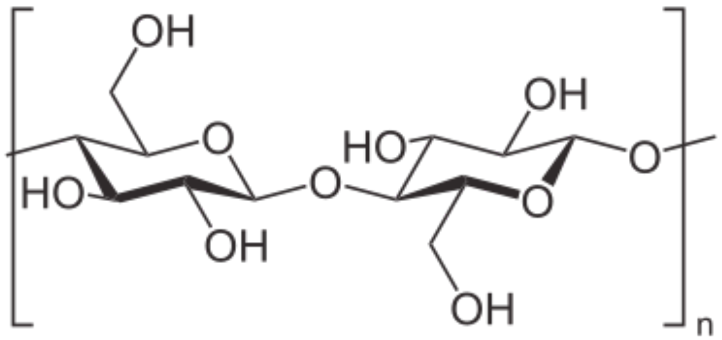
Glycogen is a polysaccharide that is physically related to amylopectin in being built only of glucose and in having a mix of alpha 1-4 and alpha 1-6 bonds. Glycogen, however, has many more alpha 1-6 branches than amylopectin, with such bonds occurring about every 10 residues. One might wonder why such branching occurs more abundantly in animals than in plants. A plausible explanation is based on the method by which these molecules are broken down. The breakdown of these polysaccharides is catalyzed by enzymes, known as **phosphorylases**, that clip glucose residues from the ends of glycogen chains and attach a phosphate to them in the process, producing **glucose-1-phosphate**. More highly branched polysaccharides have more ends to clip, and this translates to more glucose-1-phosphates that can be removed simultaneously by numerous phosphorylases. Since glucose is used for energy by muscles, glucose concentrations can be increased faster the more branched the glycogen is. Plants, which are immobile do



Glycogen Structure

from Wikipedia

not have needs for such immediate release of glucose and thus have less need for highly branched polysaccharides.



Repeating structure of cellulose

from Wikipedia

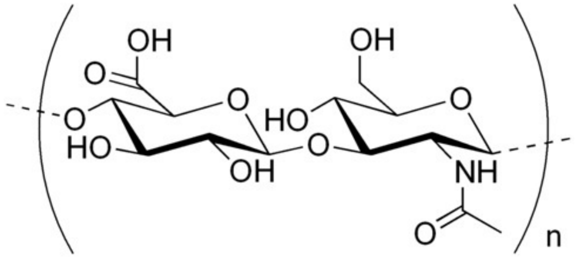
Cellulose

Another important polysaccharide containing only glucose is **cellulose**. It is a polymer of glucose used to give plant cell walls structural integrity and has the individual units joined solely in a beta 1-4 configuration. That simple structural change makes a radical difference in its digestibility. Humans are unable to break down cellulose and it passes through the digestive system as roughage. Ruminant animals, such as cattle, however have bacteria in their rumens that contain the enzyme **cellulase**. It breaks the beta 1-4 links of the glucoses in cellulose to release the sugars for energy.

Another polysaccharide used for structural integrity is known as chitin. **Chitin** makes up the exoskeleton of insects and is a polymer of a modified form of glucose known as N-acetyl-glucosamine.

Glycosaminoglycans

Yet another category of polysaccharides are the **glycosaminoglycans** (also called **mucopolysaccharides**), some examples of which include **keratan sulfate**, **heparin**, **hyaluronic acid** (right), and **chondroitin sulfate**. The polysaccharide compounds are linked to proteins, but differ from glycoproteins in having a much larger contingent of sugar residues and, further, the sugars are considerably more chemically modified. Each of them contains a repeating unit of a disaccharide that contains at least one negatively charged residue. The result is a polyanionic substance that, in its interactions with water, makes for a “slimy” feel. Glycosaminoglycans are found in snot, and in synovial fluid, which lubricates joints. Heparin is a glycosaminoglycan that helps to prevent blood from clotting.



The repeating unit in a glycosaminoglycan

from Wikipedia

Lipids and Membranes

Lipids are a broad class of molecules that all share the characteristic that they have at least a portion of them that is hydrophobic. The class of molecules includes fats, oils (and their

Hyaluronic Acid

To the tune of “*Rudolph the Red-Nosed Reindeer*”

Hyaluronic Acid
Acting almost magically
Placed just beneath the kneecap
Lubricating the debris

Better than joint replacement
Simple as 1-2-3
If it can stop the aching
You will get to keep your knee

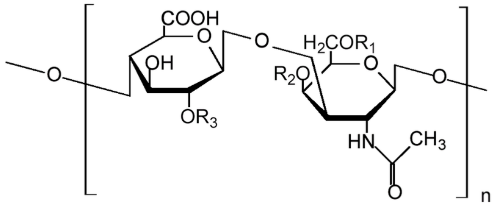
When the pain is getting bad
Try not to be sad
Just go out and have a talk
With your orthopedic doc

Beg him to use the needle
To not do so would be a crime
Hyaluronic acid
Workin’ where the sun don’t shine

H Acid

Recorded by David Simmons
Lyrics by Kevin Ahern

substituent fatty acids), steroids, fat-soluble vitamins, prostaglandins, glycerophospholipids, and sphingolipids. Interestingly, each of these can be derived from acetyl-CoA.

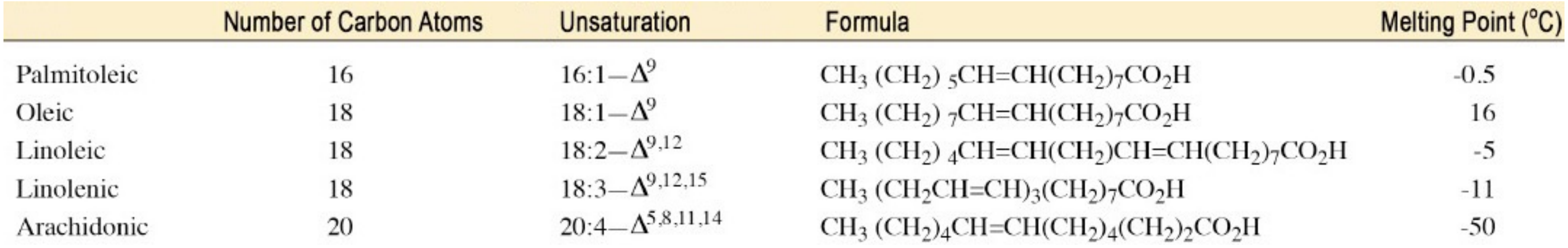


Monomeric unit of chondroitin sulfate. Chemical structure of one unit in a chondroitin sulfate chain.

Fatty Acids

Arguably, the most important lipids in our cells are the fatty acids, because they are components of all of the other lipids, except some of the steroids and fat-soluble vitamins. Consisting of a carboxyl group linked to a long aliphatic tail, fatty acids are described as either saturated (no double bonds) or unsaturated (one or more double bonds). Fatty acids with more than one double bond are described as polyunsaturated. Increasing the amount of unsaturated fatty acids (and the amount of unsaturation in a given fatty acid) in a fat decreases its melting temperature. This is also a factor in membrane fluidity. If the melting temperature of a fat is decreased sufficiently so that it is a liquid at room temperature, it is referred to as an oil. It is worth noting that organisms like fish, which live in cool environments, have fats with more unsaturation. This is why fish oil is a rich source of polyunsaturated fatty acids.

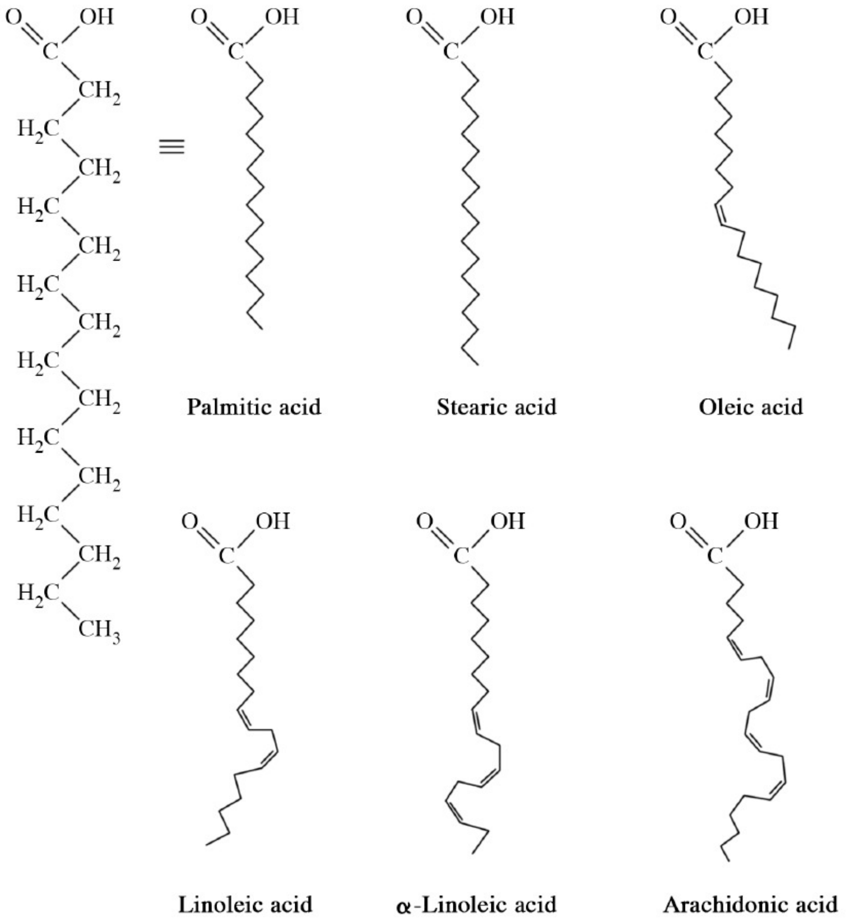
Click HERE and HERE for Kevin’s YouTube lectures on Lipids and Membranes.



	Number of Carbon Atoms	Unsaturation	Formula	Melting Point (°C)
Palmitoleic	16	$16{:}1\text{—}\Delta^{9}$	$CH_3\ (CH_2)\ _5CH{=}CH(CH_2)_7CO_2H$	-0.5
Oleic	18	$18{:}1\text{—}\Delta^{9}$	$CH_3\ (CH_2)\ _7CH{=}CH(CH_2)_7CO_2H$	16
Linoleic	18	$18{:}2\text{—}\Delta^{9,12}$	$CH_3\ (CH_2)\ _4CH{=}CH(CH_2)CH{=}CH(CH_2)_7CO_2H$	-5
Linolenic	18	$18{:}3\text{—}\Delta^{9,12,15}$	$CH_3\ (CH_2CH{=}CH)_3(CH_2)_7CO_2H$	-11
Arachidonic	20	$20{:}4\text{—}\Delta^{5,8,11,14}$	$CH_3\ (CH_2)_4CH{=}CH(CH_2)_4(CH_2)_2CO_2H$	-50

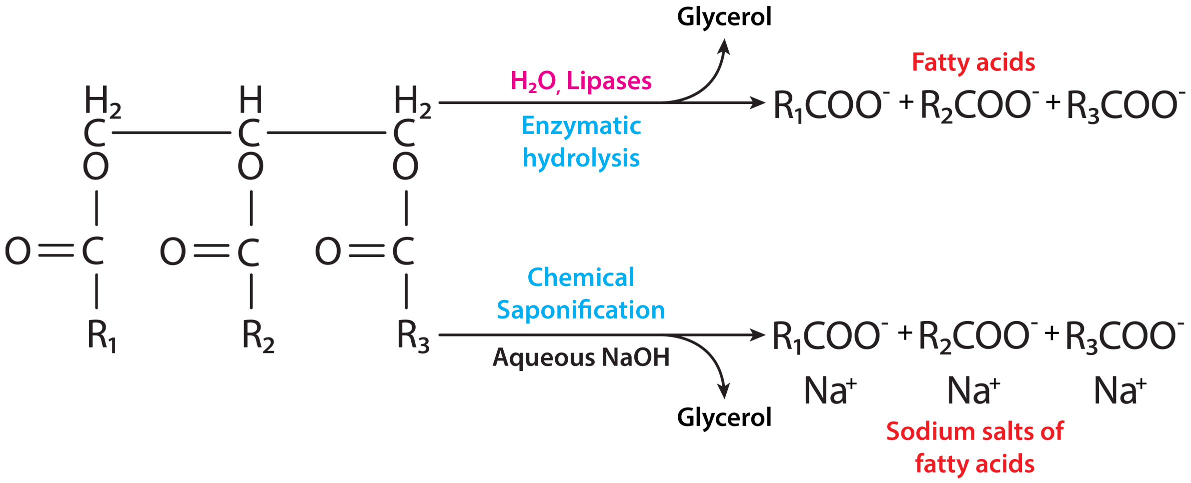
Important Unsaturated Fatty Acids

Biochemically, the double bonds found in fatty acids are predominantly in the *cis* configuration. So-called *trans* fats arise as a chemical by-product of partial hydrogenation of vegetable oil (small amounts of *trans* fats also occur naturally). In humans, consumption of trans fats raises low density lipoprotein (LDL) levels and lowers high density lipoprotein (HDL) levels. Each is thought to contribute to the risk of developing coronary artery disease. The most common fatty acids in our body include palmitate, stearate, oleate, linolenate, linoleate, and arachidonate. Fatty acids are numbered by two completely different schemes. The delta numbering scheme has the carboxyl group as #1, whereas the omega number scheme starts at the other end of the fatty acid with the methyl group as #1. Fatty acids are described as essential if they must be in the diet (can't be synthesized by the organism). Animals, including humans, cannot synthesize fatty acids with double bonds beyond position delta 9, so linoleic and linolenic acids are considered essential in these organisms.



Fatty Acids

In animal cells, fats are the primary energy storage forms. They are also known as triacylglycerols, since they consist of a glycerol molecule esterified to three fatty acids. Fats are synthesized by replacing the phosphate on phosphatidic acid with a fatty acid. Fats are stored in the body in specialized cells known as adipocytes. Enzymes known as lipases release fatty acids from fats by hydrolysis reactions. Of the various lipases acting on fat, the one that acts first, triacylglycerol lipase, is regulated hormonally.

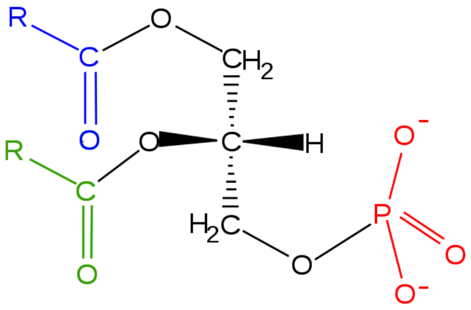

Glycerol
H₂O, Lipases
Enzymatic hydrolysis
Fatty acids
R₁COO⁻ + R₂COO⁻ + R₃COO⁻
Chemical Saponification
Aqueous NaOH
Glycerol
R₁COO⁻ + R₂COO⁻ + R₃COO⁻
Na⁺ Na⁺ Na⁺
Sodium salts of fatty acids
R₁
R₂
R₃

Hydrolysis of Fat

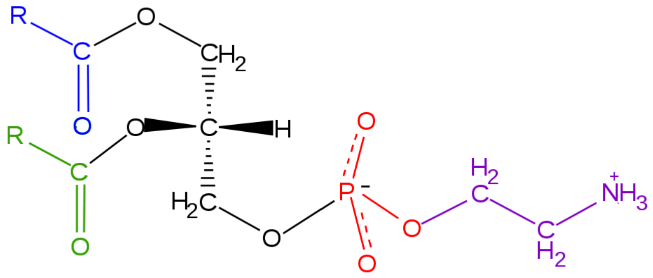
Membrane Lipids

The predominant lipids found in membranes are glycerophospholipids (phosphoglycerides) and sphingolipids. The former are related to fats structurally as both are derived from phosphatidic acid. Phosphatidic acid is a simple glycerophospholipid that is usually converted into phosphatidyl compounds. These are made by esterifying various groups, such as ethanolamine, serine, choline, inositol, and others to the phosphate. All of these compounds form lipid bilayers in aqueous solution, due to the amphiphilic nature of their structure.

Though structurally similar to glycerophospholipids, sphingolipids are synthesized completely independently of them, starting with palmitic acid and the amino acid serine. The figure on the right shows the structure of several sphingolipids. LIke the glycerophospholipids, sphingolipids are amphiphilic, but unlike them, they may have simple (in cerebrosides) or complex (in gangliosides) carbohydrates attached at one end. Most sphingolipids, except sphingomyelin, do not contain phosphate.

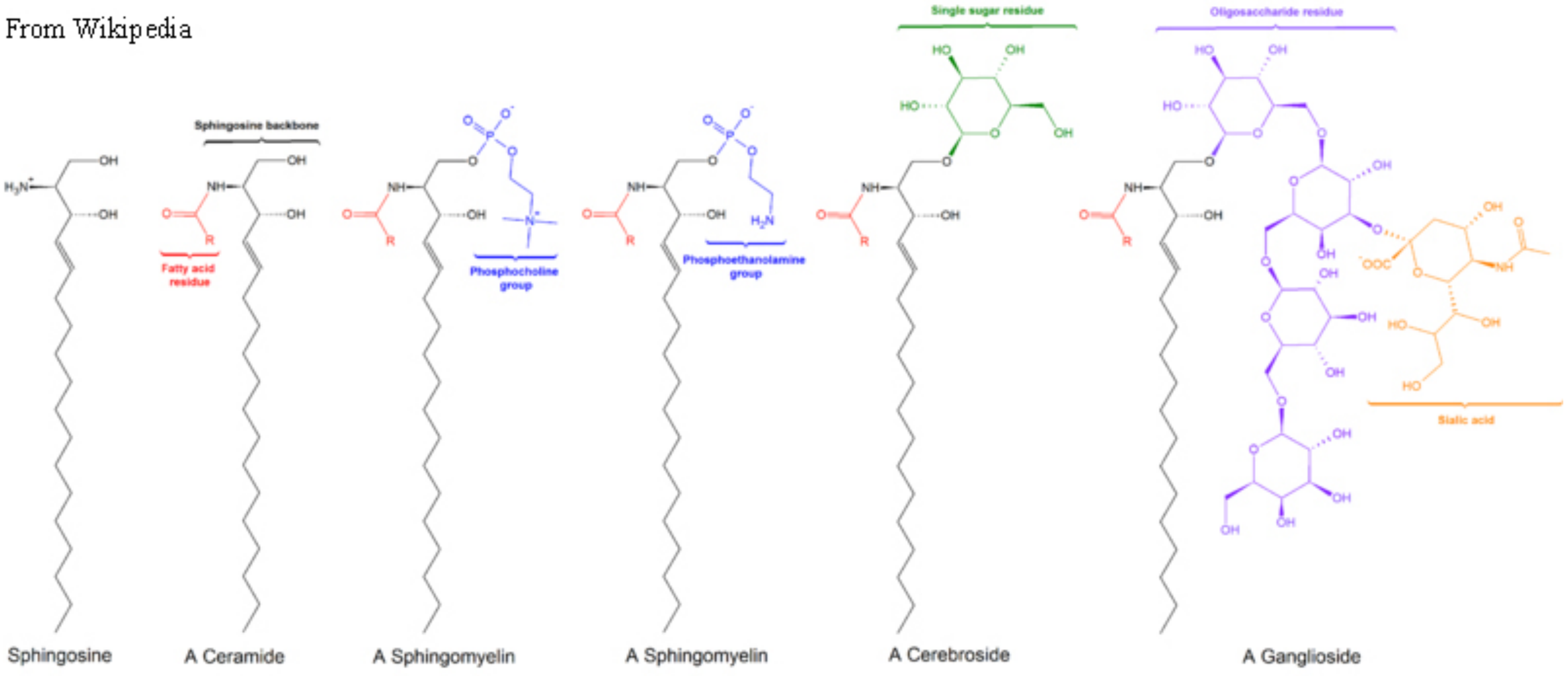

R
O
CH₂
C
O
R
O
C
H
C
O
H₂C
O
P
O⁻
O
O⁻

Phosphatidic Acid


R
O
CH₂
C
O
R
O
C
H
C
O
H₂C
O
P⁻
O
O
O
C H₂
C H₂
N⁺H₃

Phosphatidyl Ethanolamine

Steroids, such as cholesterol are also found in membranes. Cholesterol, in particular, may play an important role in membrane fluidity. Membranes can be thought of a being more “frozen” or more “fluid.” Fluidity is important for cellular membranes. When heated, membranes move from a more “frozen” character to that of a more “fluid” one as the temperature rises. The mid-point of this transition, referred to as the Tm, is influenced by the fatty acid composition of the lipid bilayer compounds. Longer and more saturated fatty acids will favor higher Tm values, whereas unsaturation and short fatty acids will favor lower Tm values. Interestingly, cholesterol does not change the Tm value, but


Sphingosine backbone
Fatty acid residue
Phosphocholine group
Phosphoethanolamine group
Single sugar residue
Oligosaccharide residue
Sialic acid
Sphingosine
A Ceramide
A Sphingomyelin
A Sphingomyelin
A Cerebroside
A Ganglioside

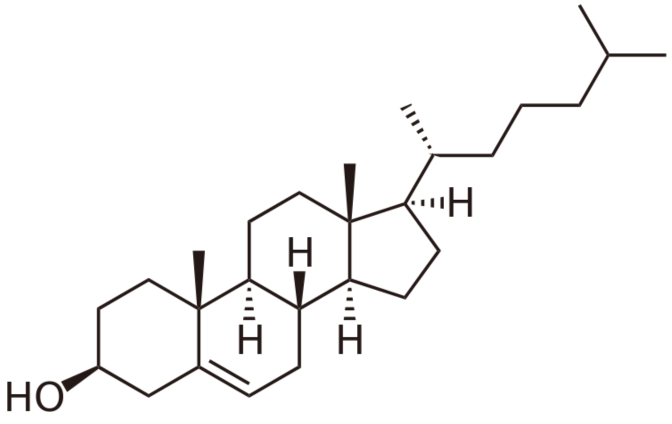
From Wikipedia

The Structures of Several Sphingolipids

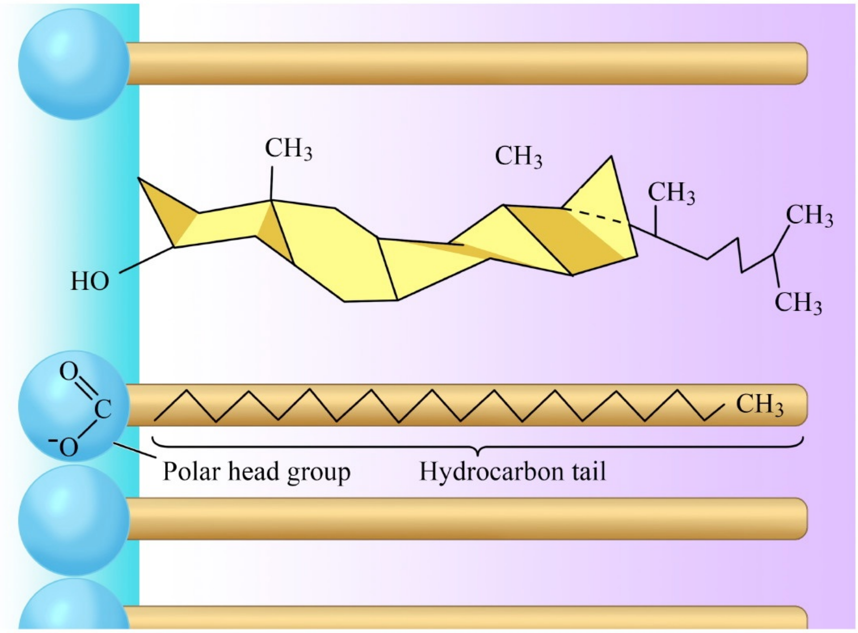
instead widens the transition range between frozen and fluid forms of the membrane.

Lipid Bilayers

The membrane around cells contains many components, including cholesterol, proteins, glycolipids, glycerophospholipids and sphingolipids. The last two of these will, in water, form what is called a lipid bilayer, which serves as a boundary for the cell that is largely impermeable to the movement of most materials across it. With the notable exceptions of water, carbon dioxide, carbon monoxide, and oxygen, most polar/ionic compounds require transport proteins to help them to efficiently navigate across the bilayer. The orderly movement of these compounds is critical for the cell to be able to 1) get food for energy; 2) export materials; 3) maintain osmotic balance; 4) create gradients for secondary transport; 5) provide electromotive force for nerve signaling; and 6) store energy in electrochemical gradients for ATP production (oxidative phosphorylation or photosynthesis). In some cases, energy is required to move the substances (active transport). In other cases, no external energy is required and they move by diffusion through specific cellular channels.



Cholesterol

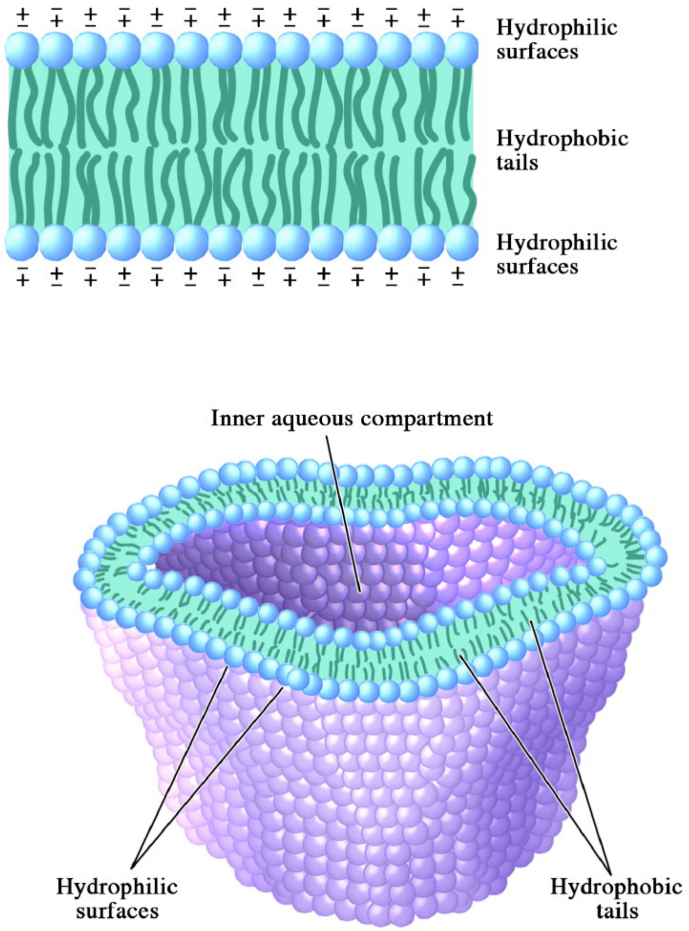

CH_3
CH_3
CH_3
CH_3
HO
CH_3
O
C
^-O
CH_3
Polar head group
Hydrocarbon tail

Cholesterol in a Lipid Bilayer

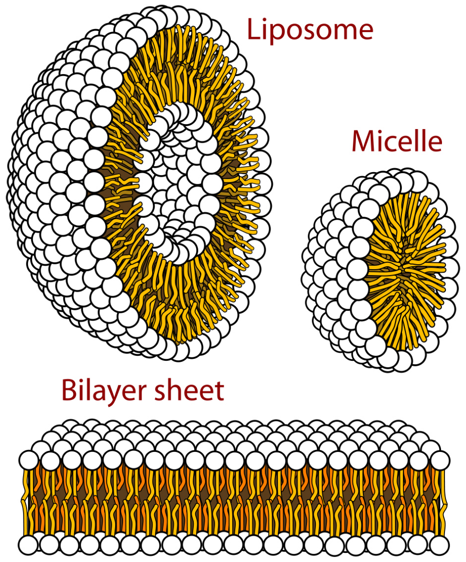
The spontaneous ability of these compounds to form lipid bilayers is exploited in the formation of artificial membranous structures called liposomes. Liposomes have some uses in delivering their contents into cells *via* membrane fusion.

Membrane Proteins

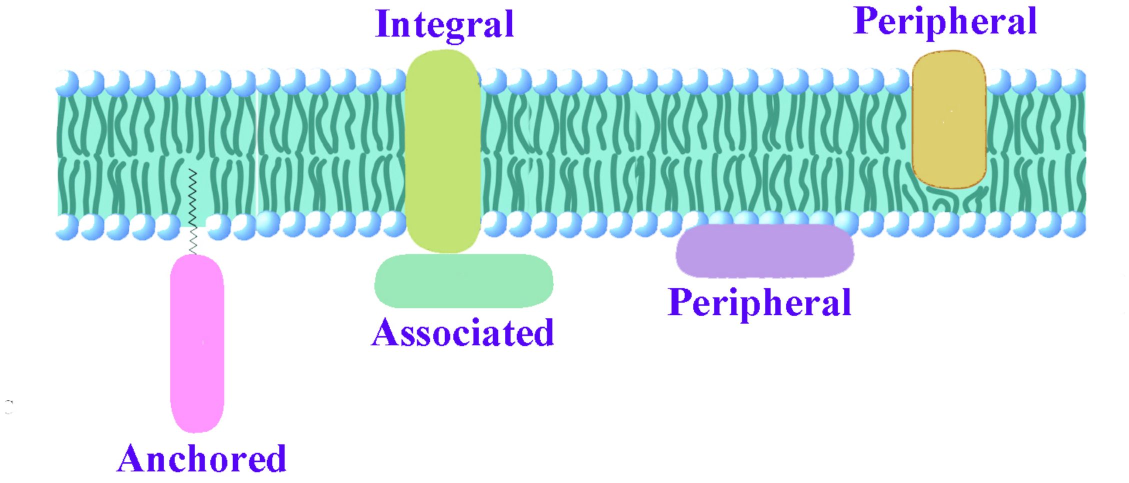
Other significant components of cellular membranes include proteins. We can put them into several categories. Integral membrane proteins are embedded in the membrane and project through both sides of the lipid bilayer. Peripheral membrane proteins are embedded in or tightly associated with part of the bilayer, but do not project completely through both sides. Associated membrane proteins are found near membranes, but may not be embedded in them. Their association may arise as a result of interaction with other proteins or molecules in the lipid bilayer. Anchored membrane proteins are not themselves embedded in the lipid bilayer, but instead are attached to a molecule (typically a fatty acid) that is embedded in the membrane.


Hydrophilic surfaces
Hydrophobic tails
Hydrophilic surfaces
Inner aqueous compartment
Hydrophilic surfaces
Hydrophobic tails

Lipid bilayer structures


Liposome
Micelle
Bilayer sheet

The geometry of the lipid bilayer is such that is hydrophobic on its interior and hydrophilic on the exterior. Such properties also dictate the amino acid side chains of proteins that interact with the



Types of Membrane Proteins

bilayer. For most membrane proteins, the polar amino acids are found where the protein projects through the bilayer (interacting with aqueous/polar substances) and the non-polar amino acids are embedded within the non-polar portion of the bilayer containing the fatty acid tails.

Glycolipids and glycoproteins play important roles in cellular identification. Blood types, for example, differ from each other in the structure of the carbohydrate chains projecting out from the surface of the glycoprotein in their membranes.

Cells have hundreds of membrane proteins and the protein composition of a membrane varies with its function and location. Mitochondrial membranes are among the most densely packed with proteins. The plasma membrane has a large number of integral proteins involved in communicating information across the membrane (signaling) or in transporting materials into the cell.

Membrane Transport

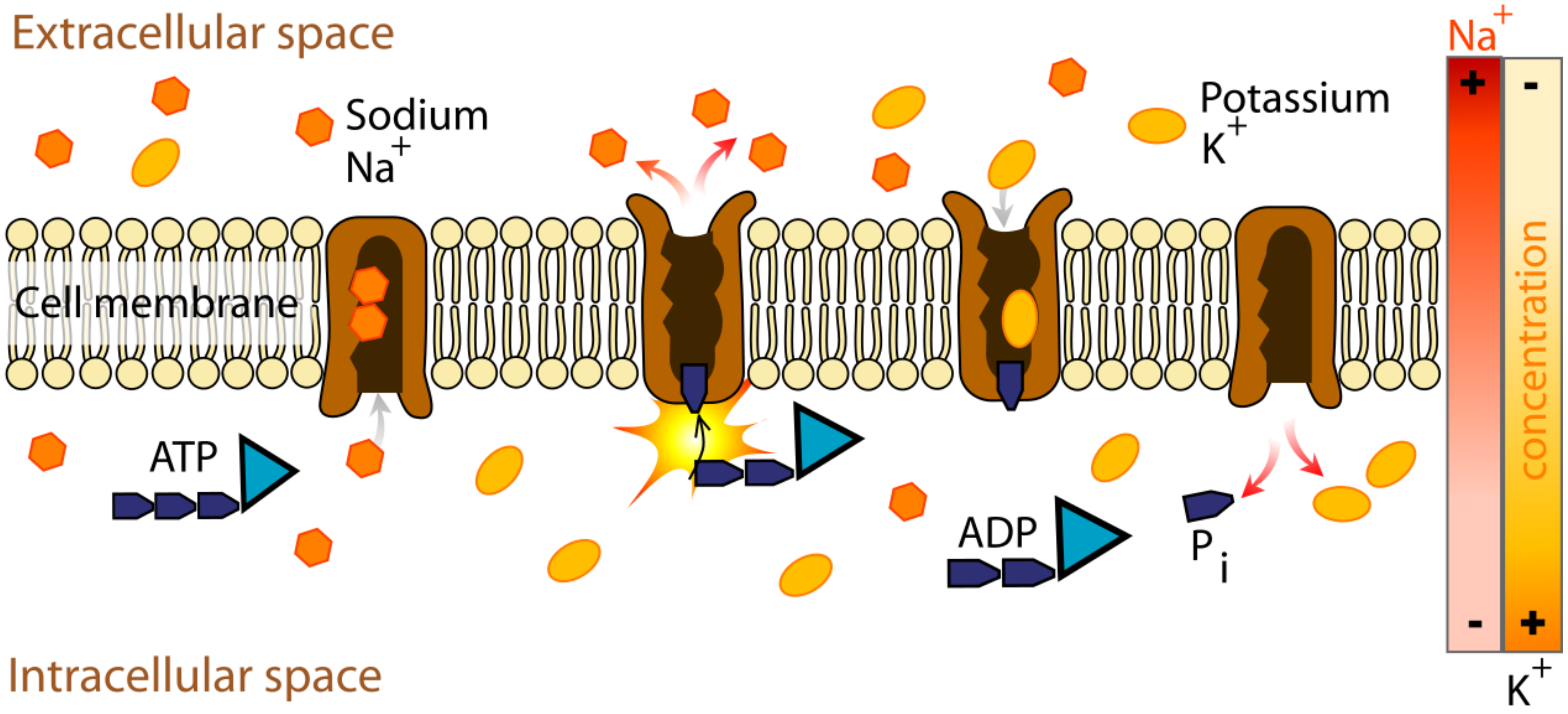
Materials, such as food and waste must be moved across a cell's lipid bilayer. There are two means of accomplishing this - passive processes and active processes. Passive processes have as their sole driving force the process of diffusion. In these systems, molecules always move from a higher concentration to a lower concentration. These can occur directly across a membrane (water, oxygen, carbon dioxide, and carbon monoxide) or through special transport proteins (glucose transport proteins of red blood cells, for example). In each case, no cellular energy is expended in the movement of the molecules. On the other hand, active processes require energy to accomplish such transport. A common energy source is ATP (see Na^+/K^+ ATPase), but many other energy sources are employed. For example, the sodium-glucose transporter uses a sodium gradient as a force for actively transporting glucose into a cell. Thus, it is important to know that not all active transport uses ATP energy. Proteins, such as the sodium-glucose transporter that move two molecules in the same direction across the membrane are called symporters (also called synporters). If the action of a protein in moving ions across a membrane results in a change in charge, the protein is described as

Click HERE and HERE for Kevin's YouTube lectures on Membrane Transport.

electrogenic and if there is no change in charge the protein is described as electro-neutral.

Na^+/K^+ ATPase

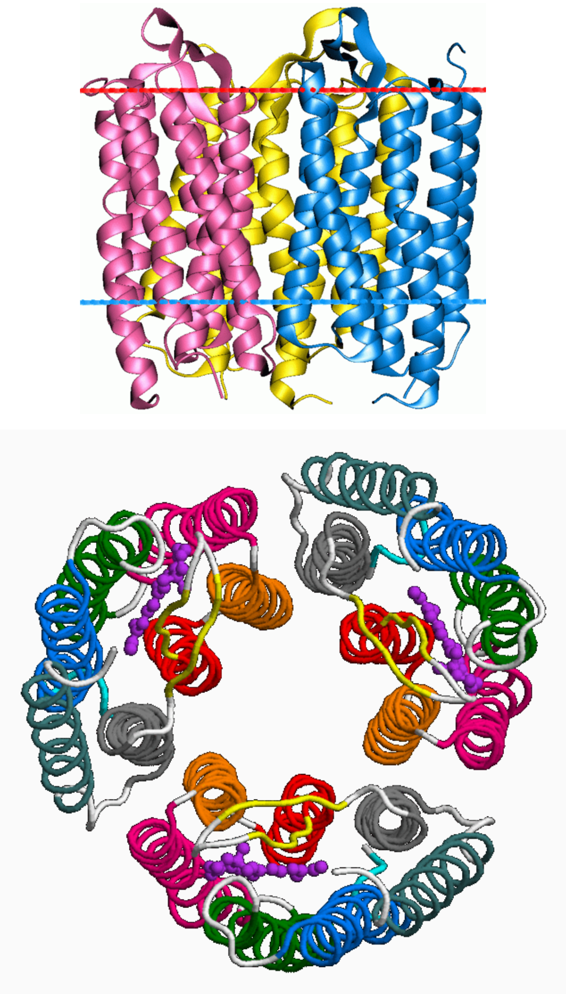
Another important integral membrane protein is the Na^+/K^+ ATPase (previous page), which transports sodium ions out of the cell and potassium ions into the cell. The protein, which is described as an anti-port (molecules moved in opposite directions across the membrane) uses the energy of ATP to create ion gradients that are important both in maintaining cellular osmotic pressure and (in nerve cells) for creating the ion gradients necessary for signal transmission. The transport system moves three atoms of sodium out of the cell and two atoms of potassium into the cell for each ATP hydrolyzed.



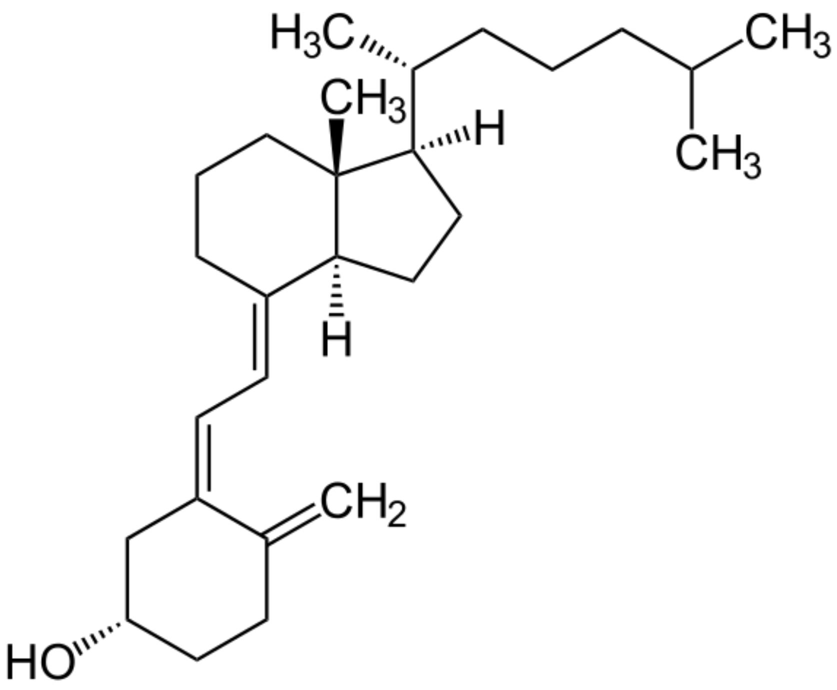
Sodium-Potassium ATPase

Bacteriorhodopsin

An interesting integral membrane protein is bacteriorhodopsin. The protein has three identical polypeptide chains, each rotated by 120 degrees relative to the others. Each chain has seven transmembrane alpha helices and contains one molecule of retinal (Vitamin A) buried deep within each cavity (shown in purple in lower figure at left). Vitamin A is light sensitive and isomerizes rapidly between a *cis* and a *trans* form in the presence of light. The changing conformation of the



Bacteriorhodopsin

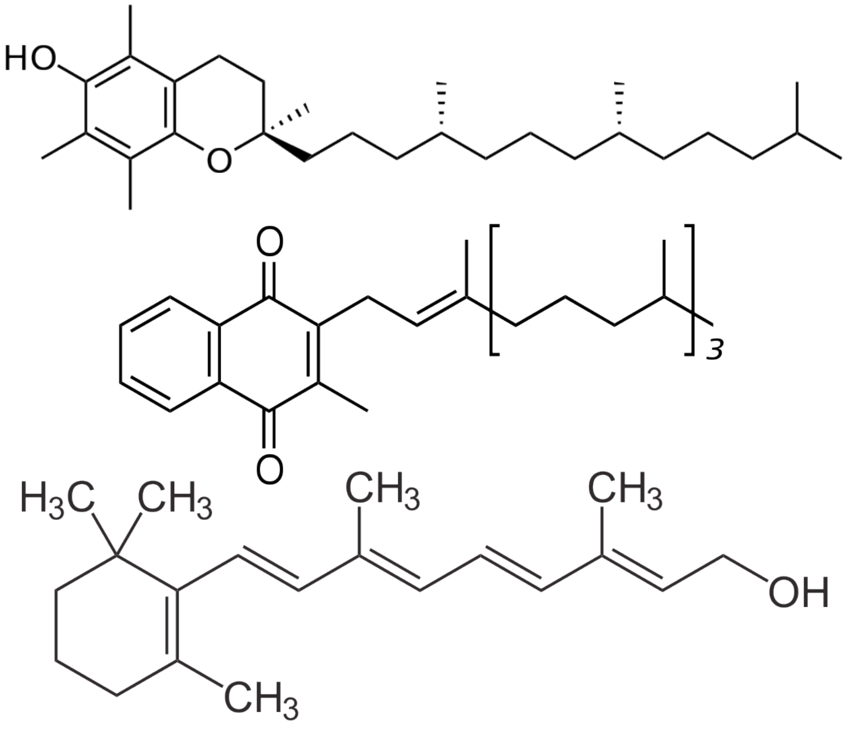


Vitamin D

vitamin A is used to transport protons through the protein and out of the bacterium, creating a proton gradient across the cell membrane, which is used ultimately to make ATP. It is not too difficult to imagine engineering an organism (say a transparent fish) to contain bacteriorhodopsin in its mitochondrial inner membrane. When light is shone upon it, the bacteriorhodopsin could be used to generate a proton gradient (much like electron transport does) and power oxidative phosphorylation. Such a fish would be partly photosynthetic in that it would be deriving energy from light, but would differ from plants in being unable to assimilate carbon dioxide in a series of "dark reactions."

Fat-Soluble Vitamins

Other lipids of note include the fat-soluble vitamins - A, D, E, and K. Vitamin A comes in three primary chemical forms, retinol (storage in liver), retinal (role in vision), and retinoic acid (roles in growth and development). Vitamin D (cholecalciferol) plays important roles in the intestinal absorption of calcium and phosphate and thus in healthy bones. Derived from ultimately from cholesterol, the compound can be synthesized in a reaction catalyzed by ultraviolet light. Vitamin E (tocopherol) is the vitamin about which the least is known. It consists of a group of eight fat-soluble compounds of which the alpha-isomer has the most biological activity. Vitamin K (the name comes from the German for coagulation vitamin) is essential for blood clotting. It is used as a co-factor for the enzyme that modifies prothrombin to increase its affinity for calcium, allowing it to be positioned closer to the site of a wound.



Top to Bottom - Vitamins E, K, and A

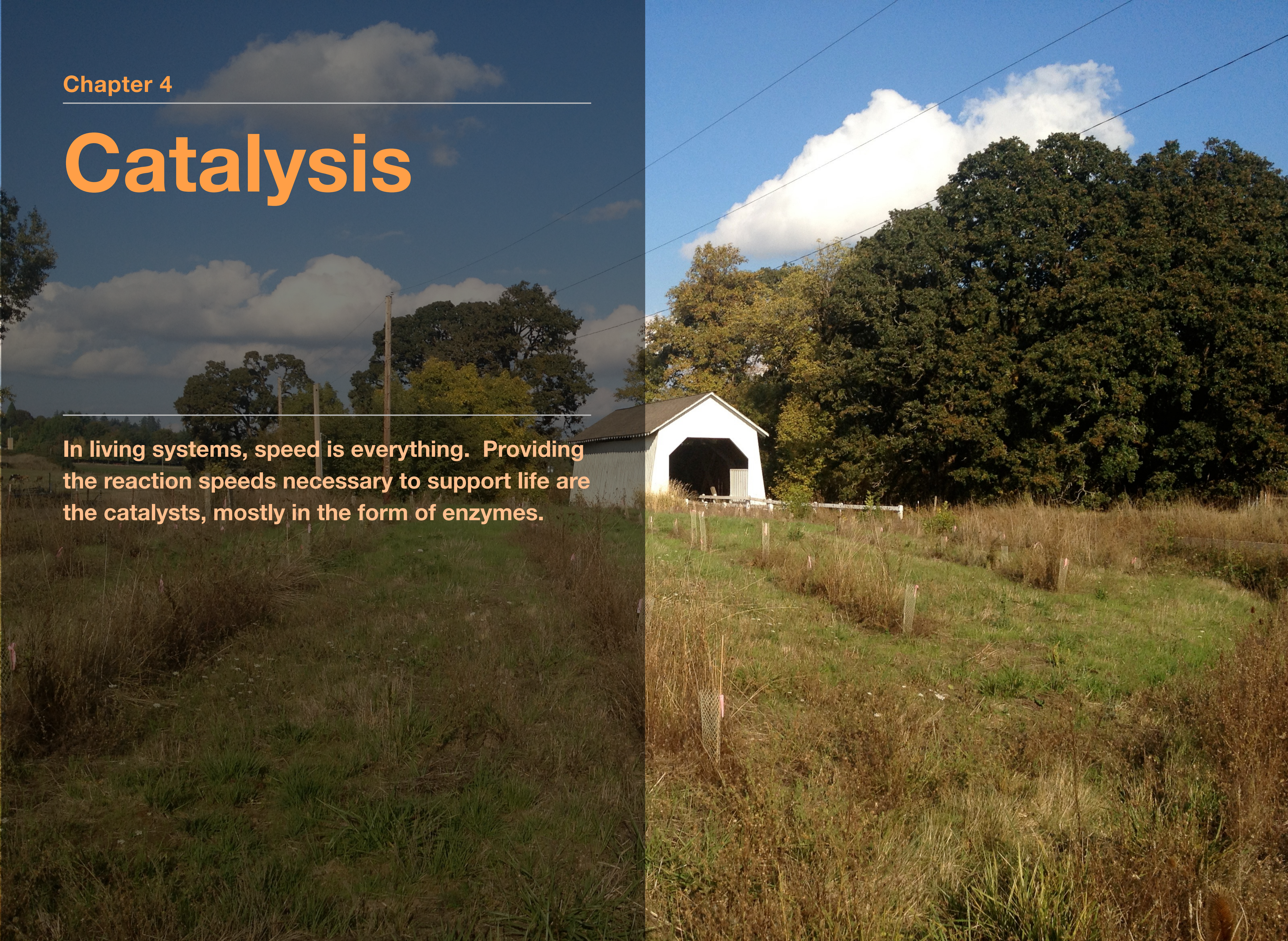
Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Chapter 4

Catalysis

In living systems, speed is everything. Providing the reaction speeds necessary to support life are the catalysts, mostly in the form of enzymes.



Catalysis

Introduction
Activation Energy
General Mechanisms of Action
Substrate Binding
Enzyme Flexibility
Active Site
Chymotrypsin
Enzyme Parameters
    V_{MAX} & K_{CAT}
    K_M
Perfect Enzymes
Lineweaver-Burk Plots
Enzyme Inhibition
    Competitive Inhibition
    No Effect On V_{MAX}
    Increased K_M
    Non-Competitive Inhibition
    Uncompetitive Inhibition
    Suicide Inhibition
Control of Enzymes
    Allosterism
    Covalent Control of Enzymes
    Other Controls of Enzymes
Ribozymes

Introduction

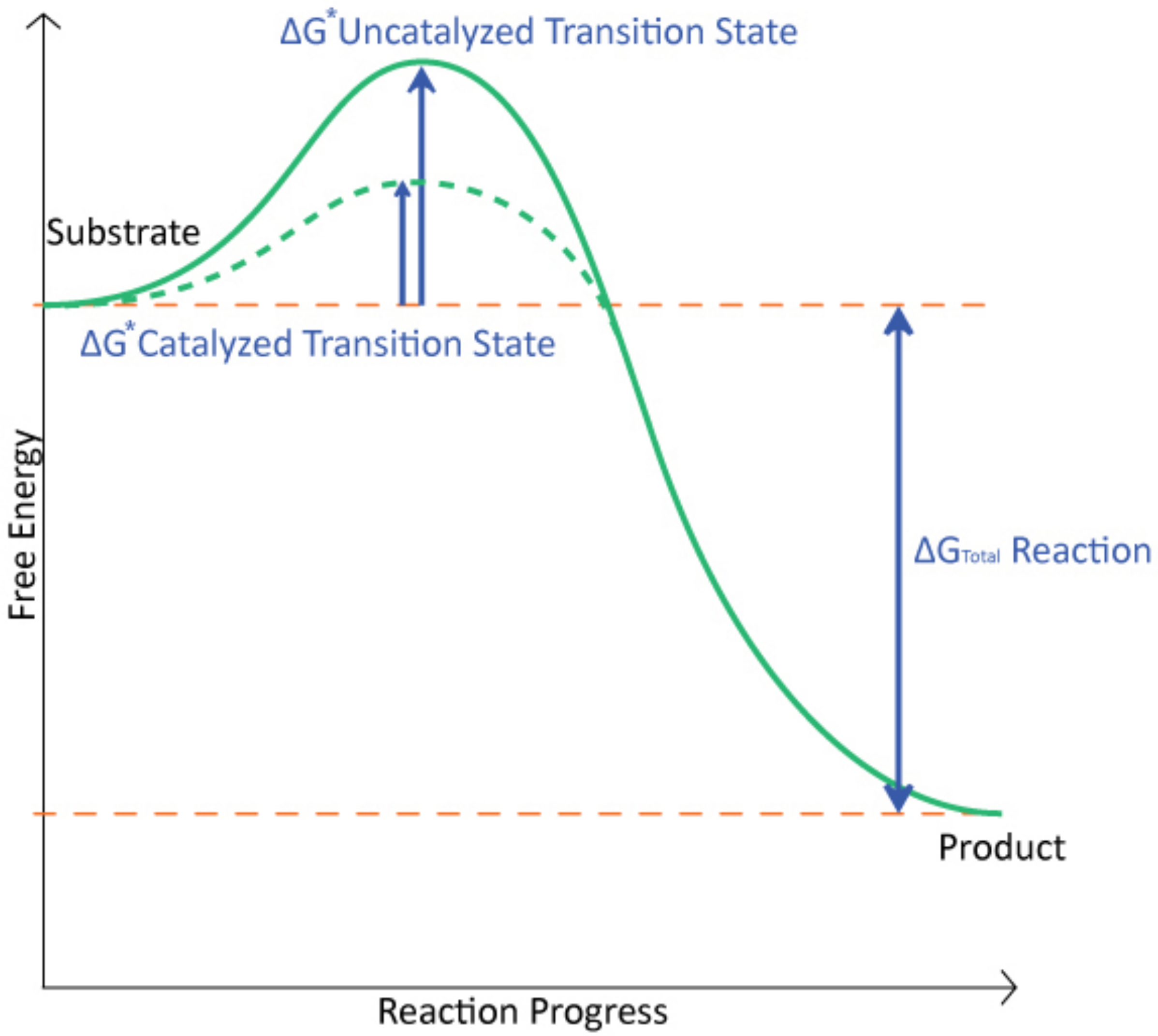
If there is a magical component to life, an argument can surely be made for it being **catalysis**. Thanks to catalysis, reactions that could take hundreds of years to complete in the "real world," occur in seconds in the presence of a catalyst. Chemical catalysts, like platinum, speed reactions, but **enzymes** (which are simply super-catalysts with a twist) put chemical catalysts to shame. To understand enzymatic catalysis, we must first understand energy. In Chapter 2, we noted the tendency for processes to move in the direction of lower energy. Chemical reactions follow this universal trend, but they often have a barrier in place that must be overcome. The secret to catalytic action is reducing the magnitude of that barrier, as we shall see.

Enzyme	Nonenzymatic Half-Life	Uncatalyzed Rate (k_{un} s^{-1})	Catalyzed Rate (k_{cat} s^{-1})	Rate Enhancement (k_{cat} s^{-1}/k_{un} s^{-1})
OMP decarboxylase	78,000,000 years	28×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Enzyme Rate Enhancements

Activation Energy

The figure below schematically depicts the energy changes that occur during the progression of a simple reaction. In the figure, the energy differences *during* the reaction are compared for a catalyzed (plot on the right) and an uncatalyzed reaction (plot on the left). Notice that the reactants start at the same energy level for both conditions and that the products end at the same energy for both as well. Thus, the difference in energy between the energy of the ending compounds and the starting compounds is the same in both cases. This is the first important rule to understand any kind of catalysis – catalysts do not change the overall energy of a reaction. Given enough time, a non-catalyzed reaction will get to the same equilibrium as a catalyzed one.


ΔG*Uncatalyzed Transition State
Substrate
ΔG*Catalyzed Transition State
Free Energy
ΔGTotal Reaction
Product
Reaction Progress

Energetic Considerations of Catalysis

Click HERE, HERE, and HERE for Kevin’s Catalytic Mechanism lectures on YouTube

Another feature to note about catalyzed reactions is the reduced energy barrier (also called the **activation energy** or free energy of activation) to reach the transition state of the catalyzed reaction. This is the second important point about catalyzed reactions – catalysts work by lowering activation energies of reactions and thus molecules more easily reach the energy necessary to get to the point where the reaction occurs. Note that these reactions are reversible. The extent to which they will proceed is a function of the size of the energy difference between the product and reactant states. The lower the energy of the products compared to the reactants, the larger the percentage of molecules that will be present as products at equilibrium. At equilibrium, of course, no change in concentration of reactants and products occurs because at this point, the forward and reverse reaction rates are the same.

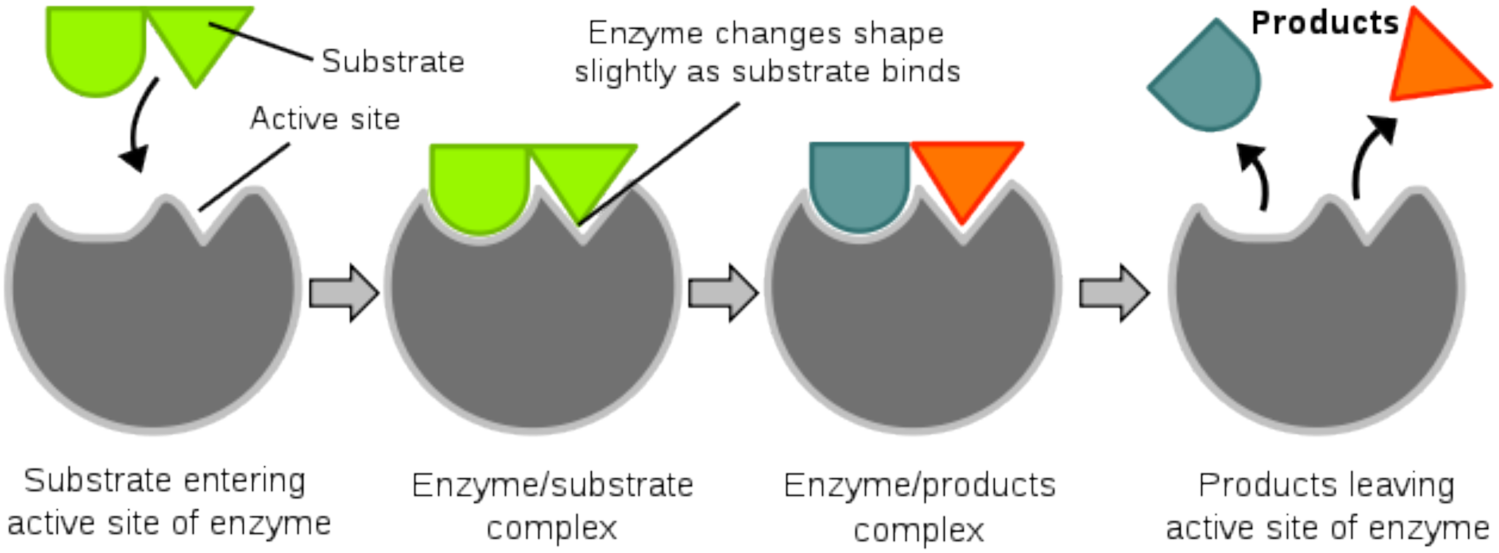
General Mechanisms of Action

As noted above, enzymes are orders of magnitude more effective (faster) than chemical catalysts. The secret of their success lies in a fundamental difference in their mechanisms of action. Every chemistry student has had hammered into their heads the fact that a catalyst speeds a reaction without being consumed by it. In other words, the catalyst ends up after a reaction just the way it started so it can catalyze other reactions, as well. Enzymes share this property, but in the middle, during the catalytic action, an enzyme is transiently changed. Such changes may be subtle electronic ones or more significant covalent modifications. It is also important to recognize that enzymes are not fixed, rigid structures, but rather are flexible. Flexibility allows movement and movement facilitates alteration of electronic environments necessary for catalysis. Enzymes are, thus, much more efficient than rigid chemical catalysts as a result of their abilities to facilitate the changes necessary to optimize the catalytic process.

Click HERE, HERE, HERE, and HERE for Kevin's enzyme catalysis lectures on YouTube

Substrate Binding

Another important difference between the mechanism of action of an enzyme and a chemical catalyst is that an enzyme has binding sites that not only 'grab' the **substrate** (molecule involved in the reaction being catalyzed), but also place it in a position to be electronically induced to react, either within itself or with another substrate. The enzyme itself may play a role in the electronic induction or the induction may occur as a result of substrates being placed in very close proximity to each other. Chemical catalysts have no such ability to bind substrates and are dependent upon them colliding in the right orientation at or near their surfaces.


Substrate
Active site
Enzyme changes shape slightly as substrate binds
Products
Substrate entering active site of enzyme
Enzyme/substrate complex
Enzyme/products complex
Products leaving active site of enzyme

Mechanism of Induced Fit

The Way They Work

To the tune of "*The Way We Were*"

Enzymes
Mighty powerhouse peptides
Cause reactions to go faster
In the cell's insides

Tiny substrates
Bring about an induced fit
Enzyme structure is affect-ed
By what binds to it

Can it be that it's just simple zen?
How the enzymes activate
If they bind effector, will they go
To an R-State, T-State?

Folding
Gives the mechanistic might
To three-D arrangement
Of the active site

Enzymes
Have a bias they can't hide
Hydrophobic side chains are
Mostly found inside

So it's the structure
For celebrating
Whenever there's debating
The way they work

T

Recorded by Liz Bacon and David Simmons
Lyrics by Kevin Ahern

Enzyme Flexibility

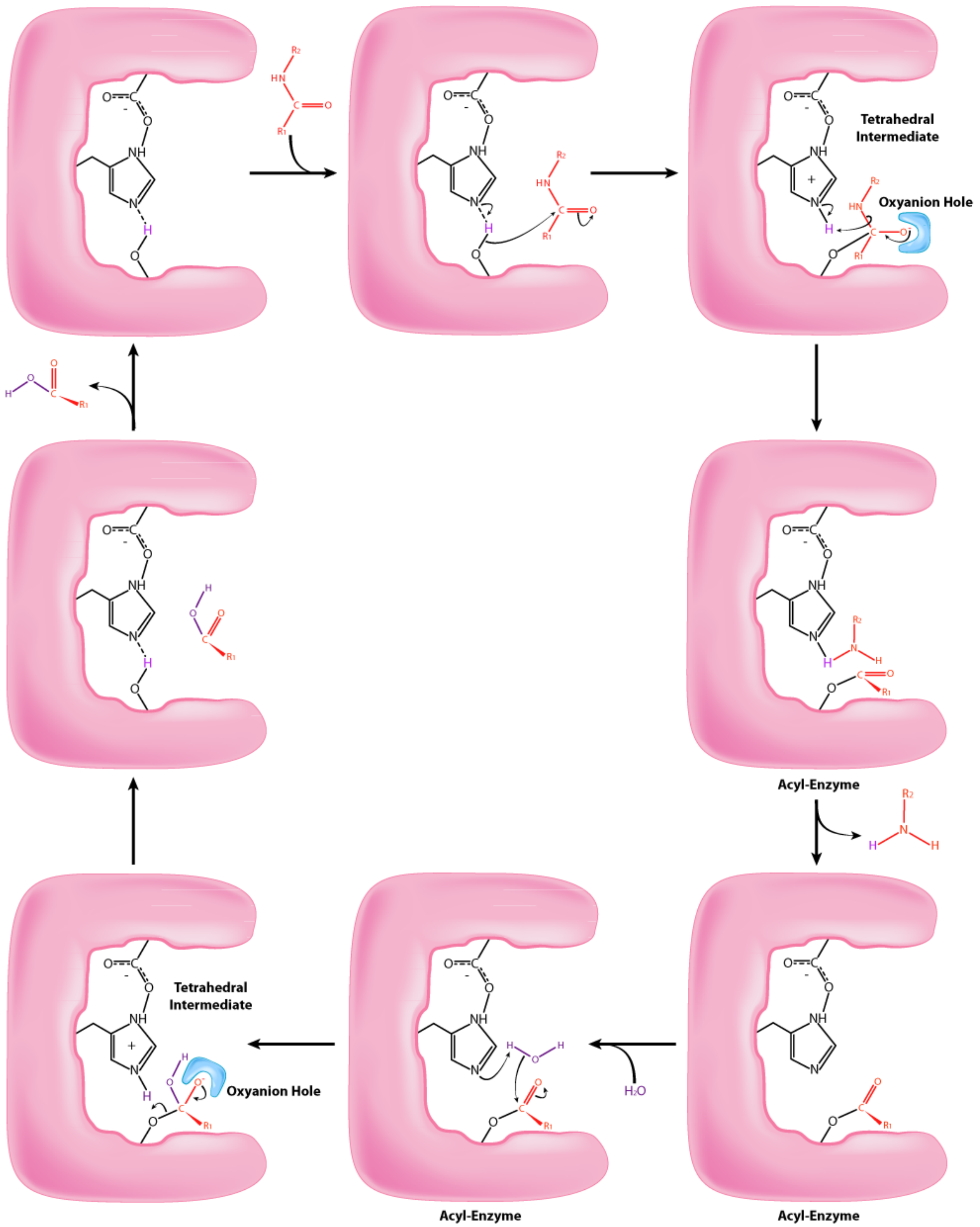
As mentioned earlier, a difference between an enzyme and a chemical catalyst is that an enzyme is flexible. Its slight changes in shape (often arising from the binding of the substrate itself) help to position substrates for reaction after they bind. These changes in shape are explained, in part, by **Koshland's Induced Fit Model of Catalysis**, which illustrates that not only do enzymes change substrates, but that substrates also transiently change enzymes. At the end of the catalysis, the enzyme is returned to its original state. Enzyme flexibility also is important for control of enzyme activity. Two distinct structures are typically described– the T (tight) state, which is a lower activity state and the R (relaxed) state, which has greater activity.

Active Site

Reactions in enzymes are catalyzed at a specific location known as the '**active site**'. Substrate binding sites are located in close physical proximity to the active site and oriented to provide access for the relevant portion of the molecule to the electronic environment of the enzyme where catalysis is initiated.

Chymotrypsin

Consider the mechanism of catalysis of the enzyme known as **chymotrypsin**. Found in our digestive system, chymotrypsin's catalytic action is cleaving peptide bonds in proteins and it uses the side chain of a serine in its mechanism of catalysis. Many other protein-cutting enzymes employ a very similar mechanism and they are known collectively as **serine proteases**. As a **protease**, it acts fairly specifically, cutting not all peptide bonds, but only those that are adjacent to specific amino acids in the protein. One of the amino acids it cuts adjacent to is phenylalanine. The enzyme's action occurs in two phases – a fast phase that occurs first and a slower phase that follows. The enzyme has a substrate binding site that includes a region of the enzyme known as the **S1 pocket**. Let us step through the mechanism by which chymotrypsin cuts adjacent to phenylalanine.



Serine Protease Mechanism

The process starts with the binding of the substrate in the S1 pocket. The S1 pocket in chymotrypsin has a hydrophobic hole in which the substrate is bound. Preferred substrates will include amino acid side chains that are hydrophobic, like phenylalanine. If an ionized side chain, like that of glutamic acid binds in the S1 pocket, it will quickly exit, much like water would avoid an oily interior. When the proper substrate binds, it stays and its presence induces an ever so slight shift in the shape of the enzyme. This

The Serine Protease Song

To the tune of “*Blackbird*”

Substrate floating in the cell’s insides
Enzyme snags it with its binding site
It supplies
Shuffling of electrons in the act to catalyze

Proteases of the serine kind
Break up peptide bonds in rapid time
Fast and slow
Steps in breaking bonds are mechanisms you should know

Asp – his - ser
Bonds beware
Inside the S1 pocket substrate sits

Alkoxides
Break peptides
Nucleophiles give bonds the fits

Peptide one exits easily
But water has to let the other flee
Bound not free
‘Cuz the enzyme’s linked to it in mechanism three

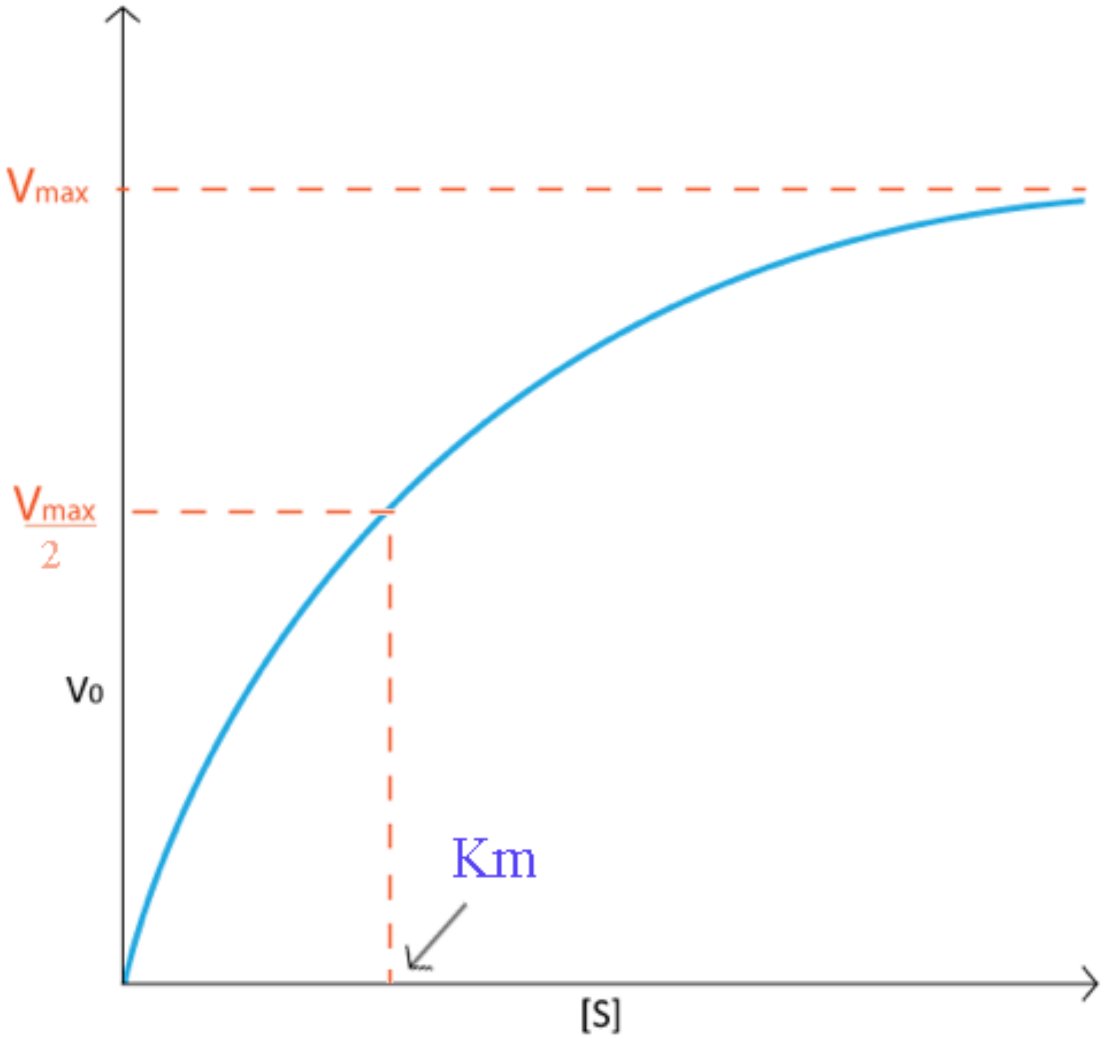
When it’s gone the enzyme’s free to catalyze you see
When it’s gone the enzyme’s free to catalyze you see

Recorded by David Simmons
Lyrics by Kevin Ahern

subtle shape change on the binding of the proper substrate starts the steps of the catalysis and is the reason that the enzyme shows specificity for cutting at specific enzyme positions in the target protein. Only amino acids with the side chains that interact well with the S1 pocket start the catalytic wheels turning.

The slight changes in shape of the enzyme upon binding of the proper substrate cause changes in the positioning of three amino acids (aspartic acid, histidine, and serine) in the active site known as the **catalytic triad**, during the second step of the catalytic action. The shift of the negatively charged aspartic acid towards the electron rich histidine ring favors the abstraction of a proton by the histidine from the hydroxyl group on the side chain of serine, resulting in production of a very reactive **alkoxide ion** in the active site. Since the active site at this point also contains the polypeptide chain positioned with the phenylalanine side chain embedded in the S1 pocket, the alkoxide ion performs a **nucleophilic attack** on the peptide bond on the carboxyl side of phenylalanine sitting in the active site. This reaction, which is the third step of catalysis, breaks the bond and causes two things to happen. First, one end of the original polypeptide is freed and exits the active site. The second is that the end containing the phenylalanine is covalently linked to the oxygen of the serine side chain. At this point we have completed the first (fast) phase of the catalysis.

The second phase of the catalysis by chymotrypsin is slower. It requires that the covalent bond between phenylalanine and serine's oxygen be broken so the peptide can be released and the enzyme can return to its original state. The process starts with entry of water into the active site. Water is attacked in a fashion similar to that of the serine side chain in the first phase, creating a reactive hydroxyl group that performs a nucleophilic attack on the phenylalanine-serine bond, releasing it and replacing the proton on serine. The second peptide is released in the process and the reaction is complete with the enzyme back in its original state.



Velocity vs Substrate Concentration Plot

Enzyme Parameters

Scientists spend a considerable amount of time characterizing enzymes. To understand how they do this and what the characterizations tell us, we must first understand a few parameters. Imagine I wished to study the reaction catalyzed by an enzyme I have just isolated. I would be interested to understand how fast the enzyme works and how much affinity the enzyme has for its substrate(s).

To perform this analysis, I would perform the following experiment. Into 20 different tubes, I would put enzyme buffer (to keep the enzyme stable), the same amount of enzyme, and then a different amount of substrate in each tube, ranging from tiny amounts in the first tubes to very large amounts in the last tubes. I would let the reaction proceed for a fixed, short amount of time and then I would measure the amount of product contained in each tube. For each reaction, I would determine the velocity of the reaction as the concentration of product found in each tube divided by the time. I would then plot the data on a graph using velocity on the Y-axis and the concentration of substrate on the X-axis.

Typically, I would generate a

curve like that shown at the left. Notice how the velocity increase is almost linear in the tubes with the lowest amounts of substrate. This indicates that substrate is limiting and the enzyme converts it into product as soon as it can bind it. As the substrate concentration increases, however, the velocity of the reaction in tubes with higher substrate concentration ceases to increase linearly and instead begins to flatten out, indicating that as the substrate concentration gets higher and higher, the enzyme has a harder time keeping up to convert the substrate to product. What is happening is the enzyme is becoming saturated with substrate at higher concentrations of the latter. Not surprisingly, when the enzyme becomes completely saturated with substrate, it will not have to wait for substrate to diffuse to it and will therefore be operating at maximum velocity.

V_{max} and K_{cat}

On a plot of Velocity versus Substrate Concentration (**V vs. [S]**), the maximum velocity (known as **V_{max}**) is the value on the Y axis that the curve asymptotically approaches. It should be noted that the value of V_{max} depends on the amount of enzyme used in a reaction. Double the amount of enzyme, double the V_{max}. If one wanted to compare the velocities of two different enzymes, it would be necessary to use the same amounts of enzyme in the different reactions they catalyze. It is desirable to have a measure of velocity that is independent of enzyme concentration. For this, we define the value **K_{cat}**, also known as the turnover number. Mathematically,

Enzyme	Turnover Number (per second)
Carbonic anhydrase	600,000
3-Ketoesteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymostrypsin	100
DNA Polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

$$K_{cat} = V_{max} /[\text{Enzyme}]$$

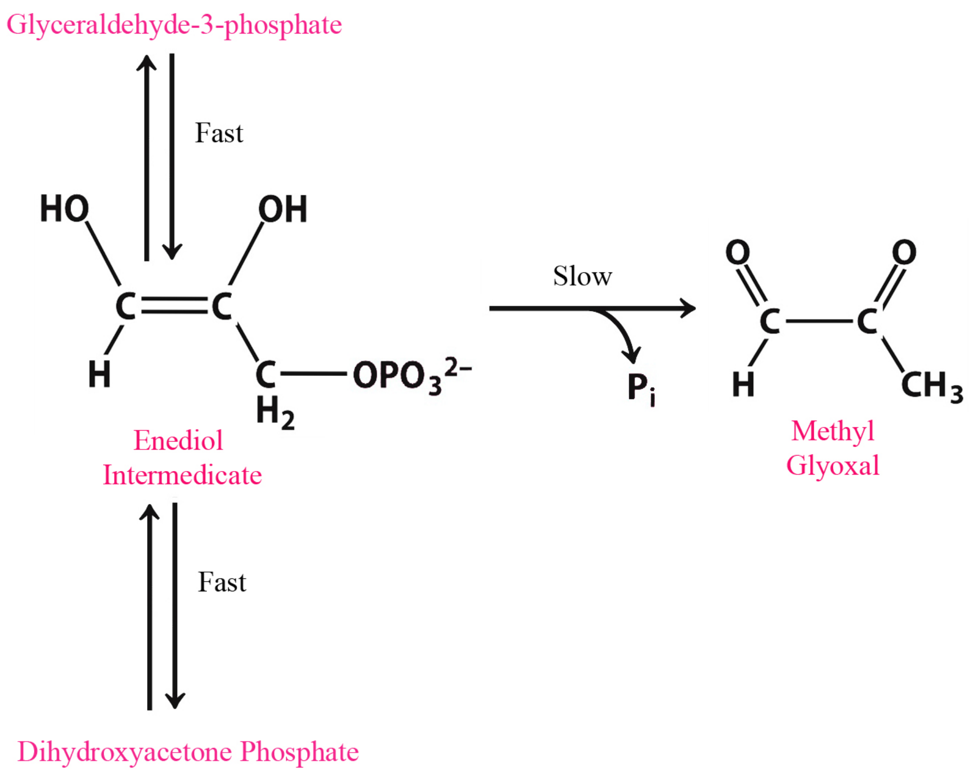
To determine K_{cat}, one must obviously know the V_{max} at a particular concentration of enzyme, but the beauty of the term is that it is a measure of velocity independent of enzyme concentration, thanks to the term in the denominator. K_{cat} is thus a constant for an enzyme under given conditions. The

Click HERE, HERE, HERE, and HERE for Kevin's Enzyme Catalysis lectures on YouTube units of K_{cat} are time^{-1}. An example would be 35/second. This would mean that each molecule of enzyme is catalyzing the formation of 35 molecules of product every second. While that might seem like a high value, there are enzymes known (**carbonic anhydrase**, for example) that have Kcat values of 10^6/second. This astonishing number illustrates clearly why enzymes seem almost magical in their action.

K_M

Another parameter of an enzyme that is useful is known as **K_M**, the Michaelis constant. What it measures, in simple terms, is the affinity an enzyme has for its substrate. Affinities of enzymes for substrates vary considerably, so knowing K_M helps us to understand how well an enzyme is suited to the substrate being used. Measurement of K_M depends on the measurement of V_{max}. On a V vs. [S] plot, K_M is determined as the x value that gives Vmax /2.

A common mistake students make in describing V_{max} is saying that K_M = V_{max} /2. This is, of course not true.



Avoidance of Formation of an Unstable Intermediate in Triose Phosphate Isomerase

K_M is a substrate concentration and is the amount of substrate it takes for an enyzme to reach V_{max} /2. On the other hand V_{max} /2 is a velocity and is nothing more than that. The value of K_M is inversely related to the affinity of the enzyme for its substrate. High values of K_M correspond to low enzyme affinity for substrate (it takes more substrate to get to V_{max}). Low K_M values for an enzyme correspond to high affinity for substrate.

Perfect Enzymes

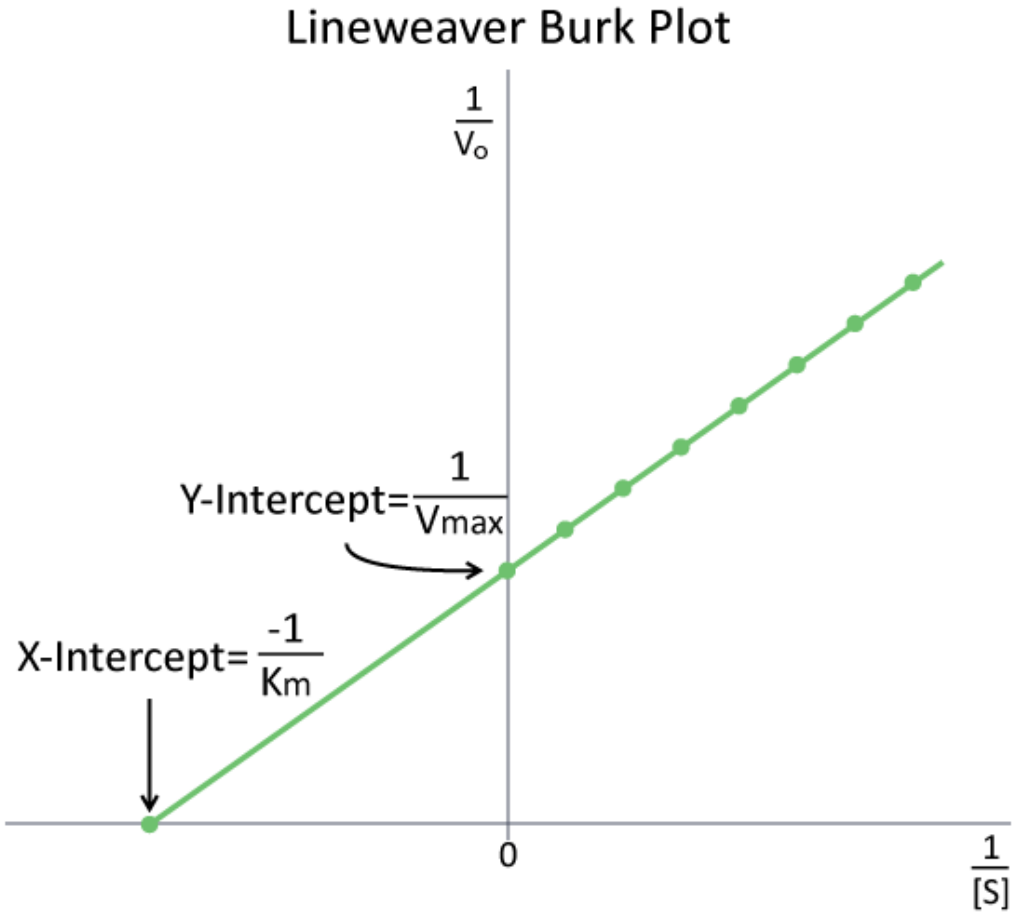
Now, if we think about what an ideal enzyme might be, it would be one that has a very high velocity and a very high affinity for its substrate. That is, it wouldn't take much substrate to get to V_{max}/2 and the K_{cat} would be very high. Such enzymes would have values of K_{cat} / K_M that are maximum. Interestingly, there are several enzymes that have this property and their maximal values are all approximately the same. Such enzymes are referred to as being “perfect” because they have reached the maximum possible value. Why should there be a maximum possible value of K_{cat} /

K_M? The answer is that movement of substrate to the enzyme becomes the limiting factor for perfect enzymes. Movement of substrate by diffusion in water has a fixed rate and that limitation ultimately determines how fast the enzyme can work. In a macroscopic world analogy, factories can’t make products faster than suppliers can deliver materials. It is safe to say for a **perfect enzyme** that the only limit it has is the rate of substrate diffusion in water.

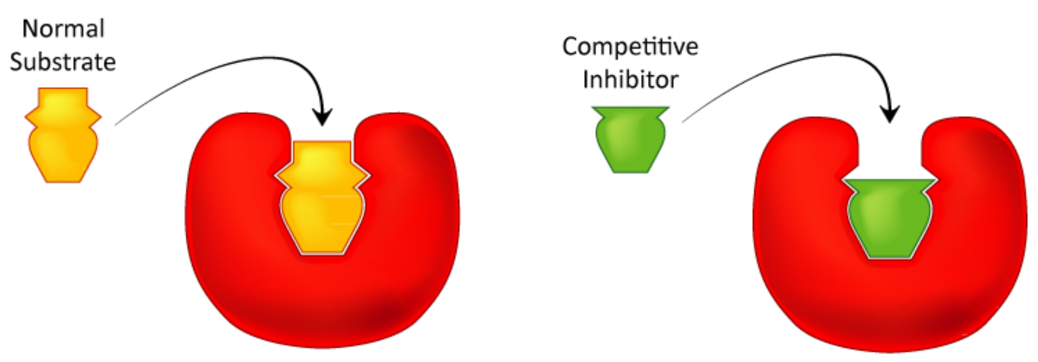
Given the “magic” of enzymes alluded to earlier, it might seem that all enzymes should have evolved to be “perfect.” There are very good reasons why most of them have not. Speed can be a dangerous thing. The faster a reaction proceeds in catalysis by an enzyme, the harder it is to control. As we all know from learning to drive, speeding causes accident. Just as drivers need to have speed limits for operating automobiles, so too must cells exert some control on the ‘throttle’ of their enzymes. In view of this, one might wonder then why any cells have evolved any enzymes to perfection. There is no single answer to the question, but a common one is illustrated by the perfect enzyme known as triose phosphate isomerase (TPI), which catalyzes a reaction in glycolysis (figure on previous page). The enzyme appears to have been selected for this ability because at lower velocities, there is breakdown of an unstable enediol intermediate that then readily forms methyl glyoxal, a cytotoxic compound. Speeding up the reaction provides less opportunity for the unstable intermediate to accumulate and fewer undesirable byproducts are made.

Lineweaver-Burk Plots

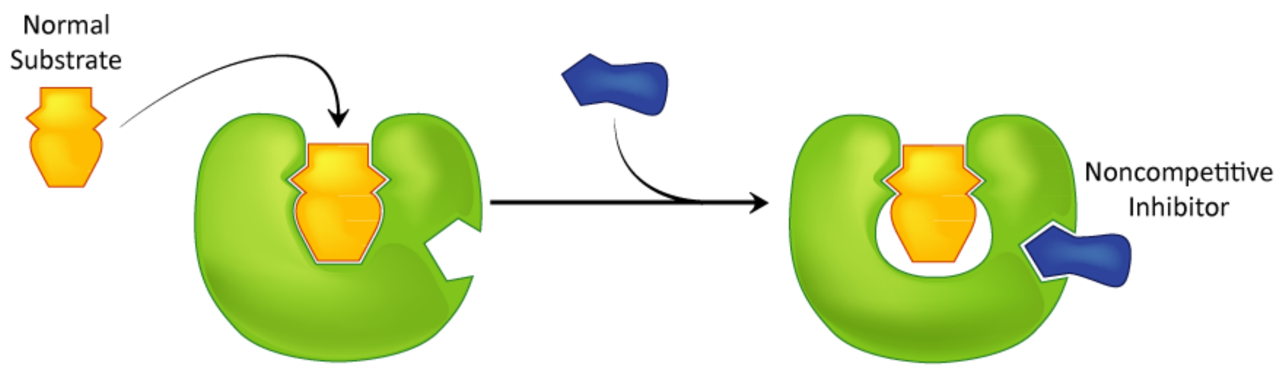
The study of enzyme kinetics is typically the most math intensive component of biochemistry and one of the most daunting aspects of the subject for many students. Although attempts are made to simplify the mathematical considerations, sometimes they only serve to confuse or frustrate students. Such is the case with modified enzyme plots, such as **Lineweaver-Burk** (left). Indeed, when presented by professors as simply another thing to



Lineweaver-Burk Plot



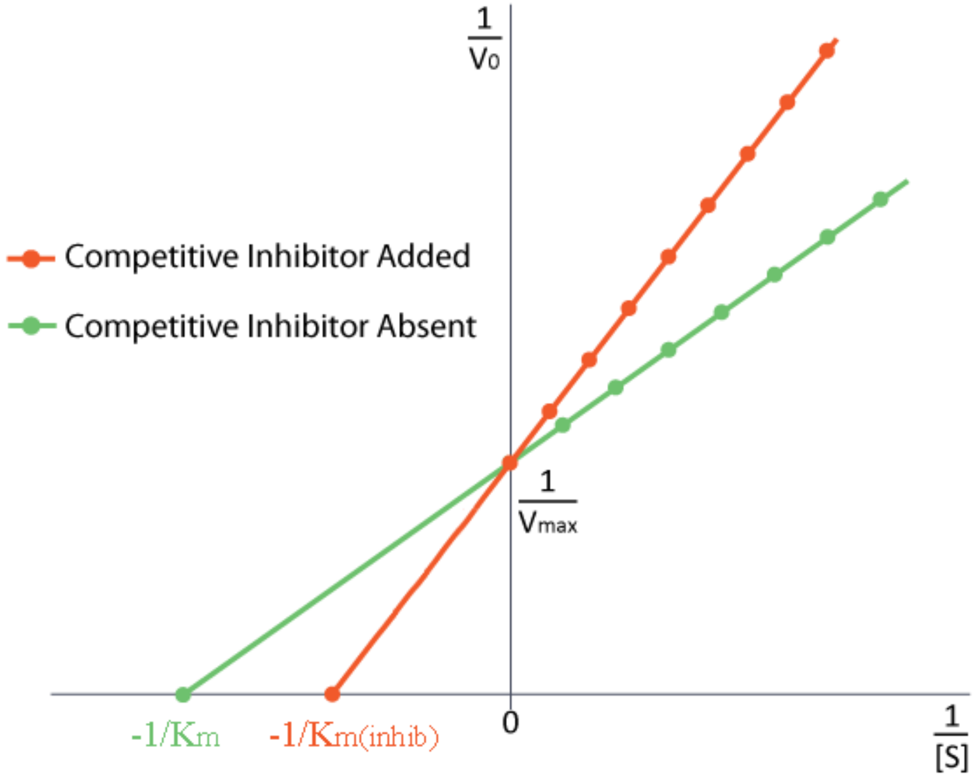
Competitive Inhibition



Non-Competitive Inhibition

memorize, who can blame students? In reality, both of these plots are aimed at simplifying the determination of parameters, such as K_M and V_{max}. In making either of these modified plots, it is important to recognize that the same data is used as in making a V vs. [S] plot. The data are simply manipulated to make the plotting easier.

For a LineWeaver-Burk, the manipulation is using the reciprocal of the values of both the velocity and the substrate concentration. The inverted values are then plotted on a graph as **1/V vs. 1/[S**]. Because of these inversions, Lineweaver-Burk plots are commonly referred to as 'double-reciprocal' plots. As can be seen at left, the value of K_M on a Lineweaver Burk plot is easily determined as the negative reciprocal of the x-intercept , whereas the Vmax is the inverse of the y-intercept. Other related manipulation of kinetic data include Eadie-Hofstee diagrams, which plot V vs V/[S] and give Vmax as the Y-axis intercept with the slope of the line being - K_M.



Lineweaver-Burk Plot of Competitive Inhibition

Enzyme Inhibition

Inhibition of specific enzymes by drugs can be medically useful. Understanding the mechanisms of enzyme inhibition is therefore of considerable importance. We will discuss four types of enzyme inhibition – competitive, non-competitive, uncompetitive, and suicide. Of these, the first three types are reversible. The last one is not.

Competitive Inhibition

Probably the easiest type of enzyme inhibition to understand is **competitive inhibition** and it is the one most commonly exploited pharmaceutically. Molecules that are competitive inhibitors of enzymes resemble one of the normal substrates of an enzyme. An example is methotrexate, which resembles the **folate** substrate of the enzyme **dihydrofolate reductase** (**DHFR**). This enzyme normally catalyzes the reduction of folate, an important reaction in the metabolism of nucleotides. When the drug **methotrexate** is present, some of the enzyme binds to it instead of to folate and during the time methotrexate is bound, the enzyme is inactive and unable to bind folate. Thus, the enzyme is inhibited. Notably, the binding site on DHFR for methotrexate is the active site, the same place that folate would normally bind. As a result, methotrexate 'competes' with folate for binding to the enzyme. The more methotrexate there is, the more effectively it competes with folate for the enzyme's active site. Conversely, the more folate there is, the less of an effect methotrexate has on the enzyme because folate outcompetes it.

Enzymes

To the tune of "*Downtown*"

Reactions alone
Could starve your cells to the bone
Thank God we all produce
Enzymes

Units arrange
To make the chemicals change
Because you always use
Enzymes

Sometimes mechanisms run like they are at the races
Witness the Kcat of the carbonic anhydrases

How do they work?
Inside of the active site
It just grabs onto a substrate
and squeezes it tight
In an
ENZYME!
CAT-al-y-sis
In an
ENZYME!
V versus S
In an
ENZYME!
All of this working for you
(Enzyme, enzyme)

Energy peaks
Are what an enzyme defeats
In its catalysis
Enzymes

Transition state
Is what an enzyme does great
And you should all know this

Enzymes

Catalytic action won't run wild - don't get hysteric
Cells can throttle pathways with an enzyme allosteric

You know it's true
So when an effector fits
It will just rearrange
all the sub-u-nits
Inside an
ENZYME!
Flipping from R to T
ENZYME!
Slow catalytically
ENZYME!
No change in Delta G
(Enzyme, enzyme)

You should relax
When seeking out the Vmax though
There are many steps
Enzymes

Lineweaver Burk
Can save a scientist work
With just two intercepts
Enzymes

Plotting all the data from kinetic exploration
Lets you match a line into a best fitting equation

Here's what you do
Both axes are inverted then
You can determine Vmax and
Establish Km
for your
ENZYMES!
Sterically holding tight
ENZYMES!
Substrates positioned right
ENZYMES!
Inside the active site
Enzymes (Enzymes, enzymes, enzymes)

Recorded by Barbara and Neal Gladstone
Lyrics by Kevin Ahern

No Effect on V_{max}

How do we study competitive inhibition? It is typically done as follows. First one performs a set of V vs. [S] reactions without inhibitor (20 or so tubes, with buffer and constant amounts of enzyme, varying amounts of substrate, equal reaction times). V vs. [S] is plotted, as well as 1/V vs. 1/[S], if desired. Next, a second set of reactions is performed in the same manner as before, except that a fixed amount of the methotrexate inhibitor is added to each tube. At low concentrations of substrate, the inhibitor competes for the enzyme effectively, but at high concentrations of substrate, the inhibitor will have a much reduced effect, since the substrate outcompetes it, due to its higher concentration (remember that the inhibitor is at fixed concentration). Graphically, the results of these experiments are shown above. Notice that at high substrate concentrations, the competitive inhibitor has essentially no effect, causing the V_{max} for the enzyme to remain unchanged. To reiterate, this is due to the fact that at high substrate concentrations, the inhibitor doesn't compete well. However, at lower substrate concentrations it does.

For inhibition, here are rules
To give to students in the schools
Non-competers muddy facts
And drop the value of V_{max}
Competers, everyone should know
Will make the K_M values grow
Uncompetition makes them think
Since both K_M and V_{max} shrink
And suicide covalently
Stops enzymes irreversibly

Increased K_M

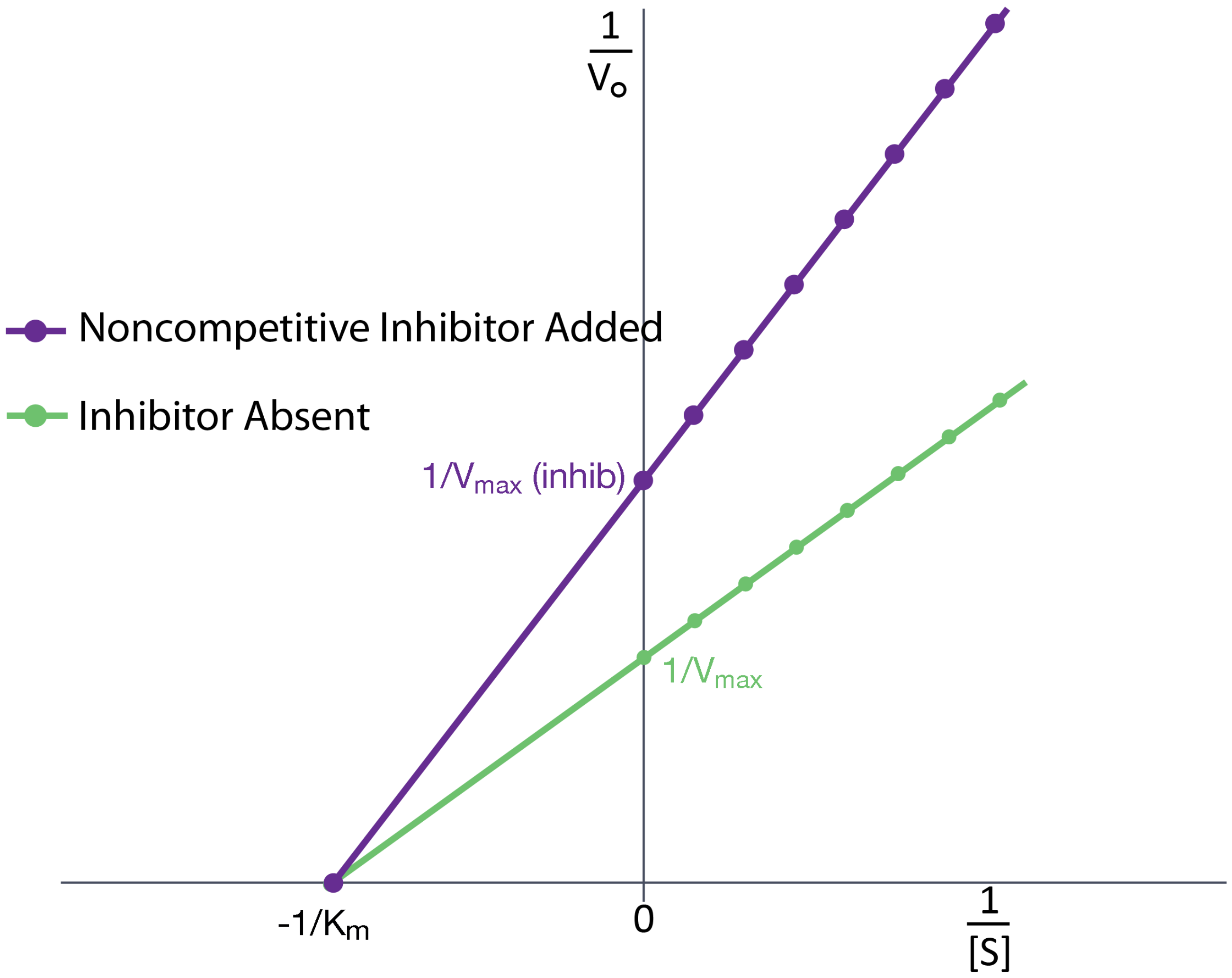
Note that the apparent K_M of the enzyme for the substrate increases ($-1/K_M$ gets closer to zero - red line above) when the inhibitor is present, thus illustrating the better competition of the inhibitor at lower substrate concentrations. It may not be obvious why we call the changed K_M the apparent K_M of the enzyme. The reason is that the inhibitor doesn't actually change the enzyme's affinity for the folate substrate. It only appears to do so. This is because of the way that competitive inhibition works. When the competitive inhibitor binds the enzyme, it is effectively 'taken out of action.' Inactive enzymes have NO affinity for substrate and no activity either. We can't measure K_M for an inactive enzyme.

The enzyme molecules that are not bound by methotrexate can, in fact, bind folate and are active. Methotrexate has no effect on them and their K_M values are unchanged. Why then, does K_M appear higher in the presence of a competitive inhibitor? The reason is that the competitive inhibitor is reducing the amount of active enzyme at lower concentrations of substrate. When the amount of enzyme is reduced, one must have more substrate to supply the reduced amount of enzyme sufficiently to get to $V_{max} / 2$.

It is worth noting that in competitive inhibition, the percentage of inactive enzyme changes drastically over the range of [S] values used. To start, at low [S] values, the greatest percentage of the enzyme is inhibited. At high [S], no significant percentage of enzyme is inhibited. This is not always the case, as we shall see in non-competitive inhibition.

Non-Competitive Inhibition

A second type of inhibition employs inhibitors that do not resemble the substrate and bind not to the active site, but rather to a separate site on the enzyme (rectangular site below). The effect of binding a non-competitive inhibitor is significantly different from binding a competitive inhibitor because there is no competition. In the case of competitive inhibition, the effect of the inhibitor could be reduced and eventually overwhelmed with increasing amounts of substrate. This was because increasing substrate made increasing percentages of the enzyme active. With **non-competitive inhibition**, increasing the amount of substrate has no effect on the percentage of enzyme that is active. Indeed, in non-competitive inhibition, the percentage of enzyme inhibited remains the same through all ranges of [S].



Lineweaver-Burk Plot of Non-Competitive Inhibition

This means, then, that non-competitive inhibition effectively reduces the amount of enzyme by the same fixed amount in a typical experiment at every substrate concentration used The effect of this inhibition is shown above. As you can see, V_{max} is reduced in non-competitive inhibition compared to uninhibited reactions. This makes sense if we remember that V_{max} is dependent on the amount of enzyme present. Reducing the amount of enzyme present reduces V_{max}. In competitive inhibition, this doesn't occur detectably, because at high substrate concentrations, there is essentially 100% of the enzyme active and the Vmax appears not to change. Additionally, K_M for non-competitively inhibited reactions does not change from that of uninhibited reactions. This is because, as noted previously, one can only measure the K_M of active enzymes and K_M is a constant for a given enzyme.

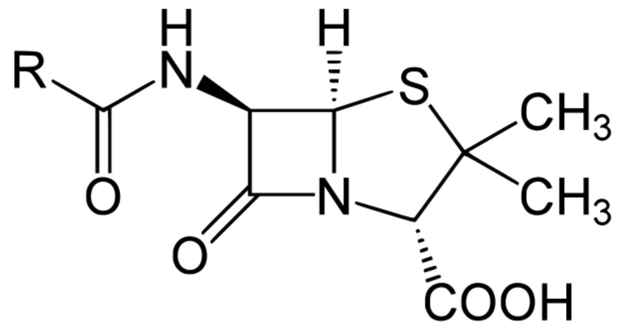
Uncompetitive Inhibition

A third type of enzymatic inhibition is that of **uncompetitive inhibition**, which has the odd property of a reduced Vmax as well as a reduced K_M. The explanation for these seemingly odd results is rooted in the fact that the uncompetitive inhibitor binds only to the **enzyme-substrate (ES) complex**. The inhibitor-bound complex forms mostly under concentrations of high substrate and the ES-I complex cannot release product while the inhibitor is bound, thus explaining the reduced V_{max}.

The reduced K_M is a bit harder to conceptualize. The answer lies in the fact that the inhibitor-bound complex effectively reduces the concentration of the ES complex. By Le Chatelier's Principle, a shift occurs to form additional ES complex, resulting in less free enzyme and more enzyme in the forms ES and ESI (ES with inhibitor). Decreases in free enzyme correspond to an enzyme with greater affinity for its substrate. Thus, paradoxically, uncompetitive inhibition both decreases Vmax and increases an enzyme's affinity for its substrate.

Suicide Inhibition

In contrast to the first three types of inhibition, which involve reversible binding of the inhibitor to the enzyme, suicide inhibition is irreversible because the inhibitor becomes covalently bound to the enzyme during the inhibition and thus cannot be removed. Suicide inhibition rather closely resembles competitive inhibition because the inhibitor generally resembles the substrate and binds to the active site of the enzyme. The primary difference is that the suicide inhibitor is chemically reactive in the active site and makes a bond with it that precludes its removal. Such a mechanism is that employed by **penicillin** (right), which covalently links to the bacterial enzyme, **D-D transpeptidase** and stops it from functioning. Since the normal function of the enzyme is to make a bond necessary for the **peptido-glycan complex** of the bacterial cell wall, the cell wall cannot properly form and bacteria cannot reproduce. If one were to measure the kinetics of suicide inhibitors under conditions where there was more enzyme than inhibitor, they would resemble non-competitive inhibition's kinetics because both involve reducing the amount of active enzyme by a fixed amount in a set of reactions.



Penicillin

Control of Enzymes

It is appropriate that we talk at this point about mechanisms cells use to control enzymes. There are four general methods that are employed. They include 1) allosterism; 2) **covalent modification**; 3) access to substrate; and 4) control of enzyme synthesis/breakdown. Some enzymes are controlled by more than one of these methods.

Allosterism

The term allosterism refers to the fact that the activity of certain enzymes can be affected by the binding of small molecules to the enzyme. In **allostery**, the molecules that are binding are non-substrate molecules that bind at a place on the enzyme other than the active site.

Catalyze

To the tune of "*Close to You*"

My enzymes
Truly are inclined
To convert
Things they bind
Turn the key
Covalently
Cat-a-lyze

How do cells
Regulate these roles?
Allo-ster
-ic controls
Two forms, you see
States R and T
Mod-u-late

Competing inhibition keeps
The substrates from the active site
They raise Km, but leave Vmax and shirk
While the non-competers bind elsewhere
And lift the plot made on Lineweaver-Burk

Other ways
Enzymes can be blocked
When things bind
Then get locked
Stuck not free
Tied to the key
Su-i-cid

Penicillin's action stops
Peptidoglycan cross-links in
Bacterial cell walls in awesome ways
Beta lactam ring's reactive site
Starts bonding with D-D-transpeptidase

So there are
Several enzyme states
Counteract
-ing substrates
Now you see
Blocking the key
Regulates

Cat-a-lysts
Have to be controlled
Some get slowed
Put on hold
It's sublime
How the enzymes
(slow) Cat-a-lyze

It's sublime
How the enzymes
(slow) Cat-a-lyze

Recorded by Barbara and Neal Gladstone
Lyrics by Kevin Ahern

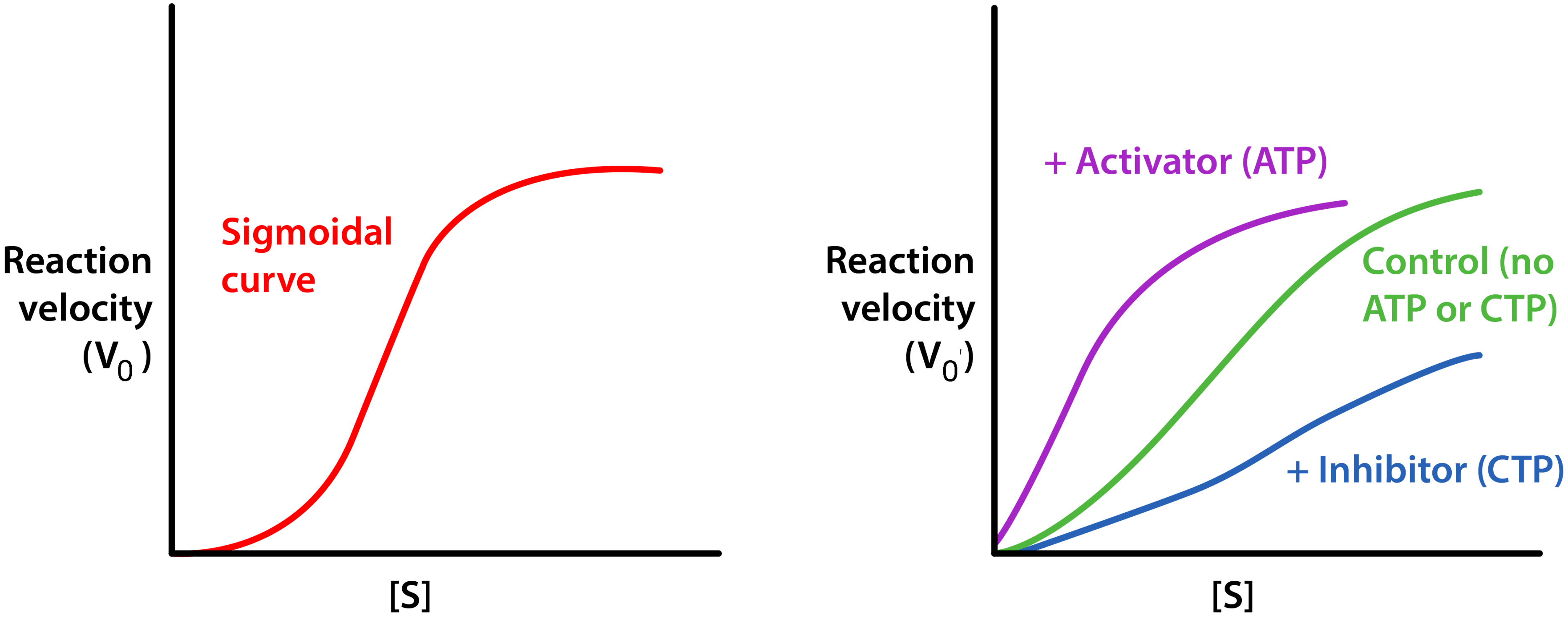
An excellent example of allosteric control is the regulation of **HMG-CoA reductase**, which catalyzes an important reaction in the pathway leading to the synthesis of cholesterol. Binding of **cholesterol** to the enzyme reduces the enzyme's activity significantly. Cholesterol

Click HERE, HERE, and HERE for Kevin's Enzyme Regulation lectures on YouTube

is not a substrate for the enzyme, but, notably, is the end-product of the pathway that HMG-CoA catalyzes a reaction in. When enzymes are inhibited by an end-product of the pathway in which they participate, they are said to be feedback inhibited.

Feedback inhibition always operates by allosterism and further, provides important and efficient control of an entire pathway. By inhibiting an early enzyme in a pathway, the flow of materials for the entire pathway is stopped or reduced, assuming there are not alternate supply methods. In the cholesterol biosynthesis pathway, stopping this one enzyme has the effect of shutting off (or at least slowing down) the entire pathway.

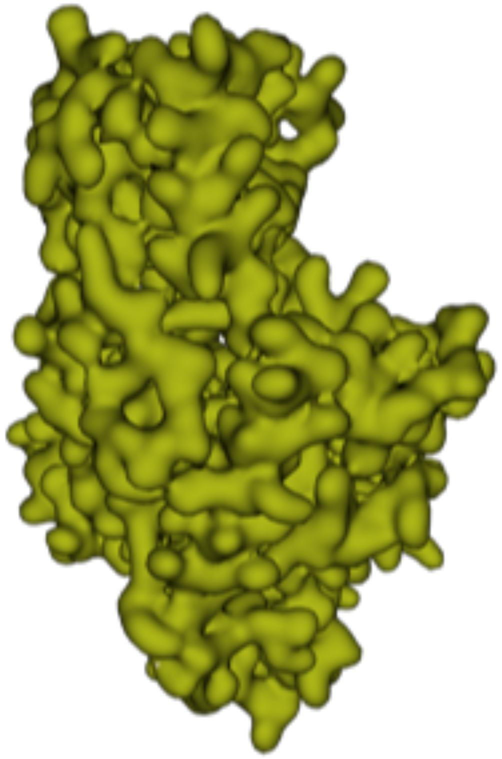
Another excellent example is the enzyme **aspartate transcarbamoylase** (**ATCase**), which catalyzes an early reaction in the synthesis of pyrimidine nucleotides. This enzyme has two allosteric effectors, ATP and CTP, that are not substrates and that bind at a regulatory site on the enzyme that is apart from the catalytic, active site. CTP, which is the end-product of the pathway, is a feedback inhibitor of the enzyme. ATP, on the other hand, acts to activate the enzyme when it binds to it.



Allosteric Effects on ATCase

Allosterically, regulation of these enzymes works by inducing different physical states (shapes, as it were) that affect their ability to bind to substrate. When an enzyme is inhibited by binding an effector, it is converted to the T (also called tight) state, it has a reduced affinity for substrate and it is through this means that the reaction is slowed. On the other hand, when an enzyme is activated by effector binding, it converts to the R (relaxed) state and binds substrate much more readily. When no effector is present, the enzyme may be in a mixture of T and R state. The V vs. S plot of allosteric enzymes resembles the oxygen binding curve of hemoglobin (see HERE). Even though hemoglobin is not an enzyme and is thus not catalyzing a reaction, the similarity of the plots is not coincidental. In both cases, the binding of an external molecule is being measured – directly by the hemoglobin plot and indirectly by the enzyme plot, since substrate binding is a factor in enzyme reaction velocity.

Interactive 4.1



Hexokinase - Not Bound to Substrate

Covalent Control of Enzymes

Some enzymes are synthesized in a completely inactive form and their activation requires covalent bonds in them to be cleaved. Such inactive forms of enzymes are called zymogens. Examples include the proteins involved in blood clotting and proteolytic enzymes of the digestive system, such as trypsin, chymotrypsin, and others. The zymogenic forms of these enzymes are known as trypsinogen and chymotrypsinogen, respectively. Synthesizing some enzymes in an inactive form makes very good sense when an enyzme’s activity might be harmful to the tissue where they are being made. For example, the painful condition known as pancreatitis arises when digestive enzymes made in the pancreas are activated too soon and end up attacking the pancreas.

Blood clotting involves polymerization of a protein known as fibrin. Since random formation of fibrin is extremely hazardous (heart attack/stroke), the body synthesizes fibrin as a zymogen (fibrinogen) and its activation results from a “cascade” of activations of proteases that arise when a signal is received from a wound. Similarly, removal of fibrin clots is also controlled by a zymogen (plasminogen), since random clot removal would also be hazardous.

Another common mechanism for control of enzyme activity by covalent modification is phosphorylation. The phosphorylation of enzymes (on the side chains of serine, threonine or tyrosine residues) is carried out by protein kinases. Enzymes activated by phosphorylation can be regulated by the addition of phosphate groups by kinases or their removal by phosphatases.

Other Enzyme Control Mechanisms

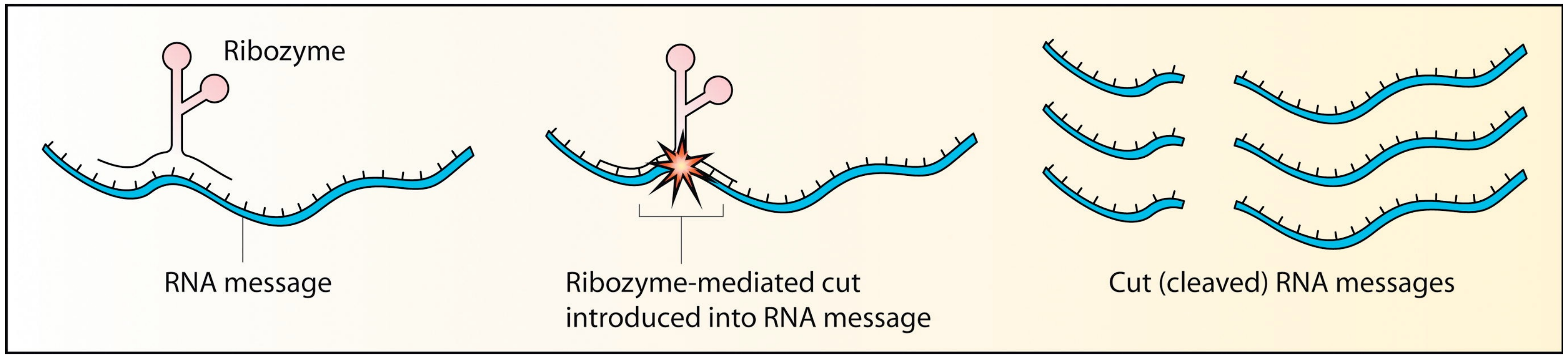
Other means of controlling enzymes relate to access to substrate (substrate-level control) and control of enzyme synthesis. Hexokinase is an enzyme that is largely regulated by availability of its substrate, glucose. When glucose concentration is low, the product of the enzyme's catalysis, glucose-6-phosphate, accumulates and inhibits the enzyme's function.

Regulation of enzymes by controlling their synthesis is covered later in the book in the discussion relating to control of gene expression.

Ribozymes

Proteins do not have a monopoly on acting as biological catalysts. Certain RNA molecules are also capable of speeding reactions. The most famous of these molecules was discovered by Tom Cech in the early 1980s Studying excision of an intron in *Tetrahymena*, Cech was puzzled at his inability to find any proteins catalyzing the process. Ultimately, the catalysis was recognized as coming from the intron itself. It was a self-splicing RNA and since then, many other examples of catalytic RNAs capable of cutting other RNAs have been found.

Ribozymes, however, are not rarities of nature. The protein-making ribosomes of cells are essentially giant ribozymes. The 23S rRNA of the prokaryotic ribosome and the 28S rRNA of the eukaryotic ribosome catalyze the formation of peptide bonds. Ribozymes are also important in our understanding of the evolution of life on Earth. They have been shown to be capable *via* selection to evolve self-replication. Indeed, ribozymes actually answer a chicken/egg dilemma - which came first, enzymes that do the work of the cell or nucleic acids that carry the information required to produce the enzymes. As both carriers of genetic information and catalysts, ribozymes are likely both the chicken *and* the egg in the origin of life.

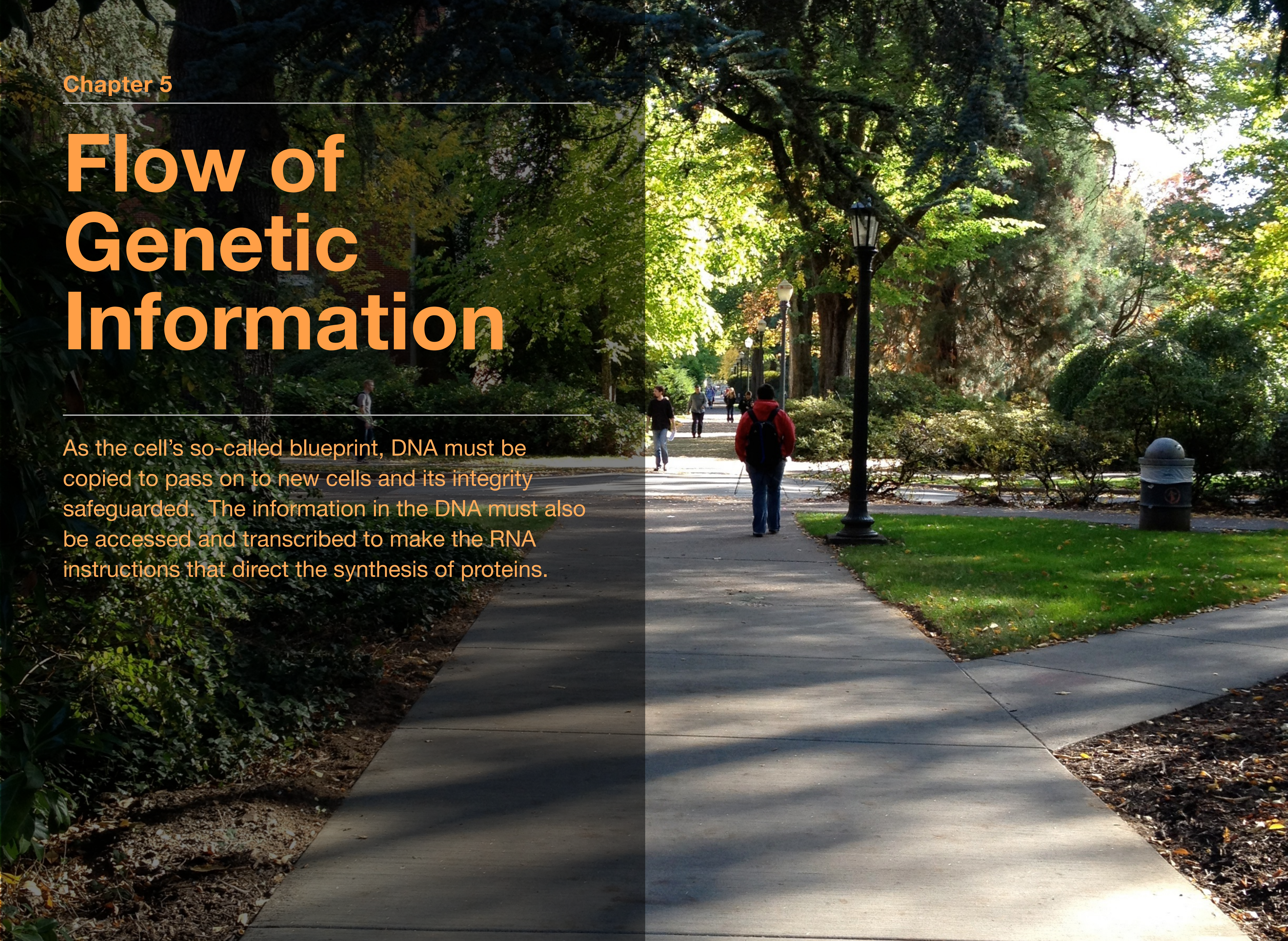


Ribozyme Catalytic Action

from Wikipedia

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12



Chapter 5

Flow of Genetic Information

As the cell's so-called blueprint, DNA must be copied to pass on to new cells and its integrity safeguarded. The information in the DNA must also be accessed and transcribed to make the RNA instructions that direct the synthesis of proteins.

Flow of Genetic Information

DNA Replication

DNA Repair

Post-Replicative Mismatch Repair

Systems to Repair Damage to DNA

- **Nucleotide Excision Repair**
- **Base Excision Repair**

Transcription

Regulation of Transcription

RNA Processing

Translation

DNA Replication

The only way to make new cells is by the division of pre-existing cells. This means that all organisms depend on cell division for their continued existence. DNA, as you know, carries the genetic information that each cell needs. Each time a cell divides, all of its DNA must be copied faithfully so that a copy of this information can be passed on to the daughter cell. This process is called DNA replication.

Before examining the actual process of DNA replication, it is useful to think about what it takes to accomplish this task successfully.

Consider the challenges facing a cell in this process:

•The sheer number of nucleotides to be copied is enormous: e.g., in human cells, on the order of several billion.

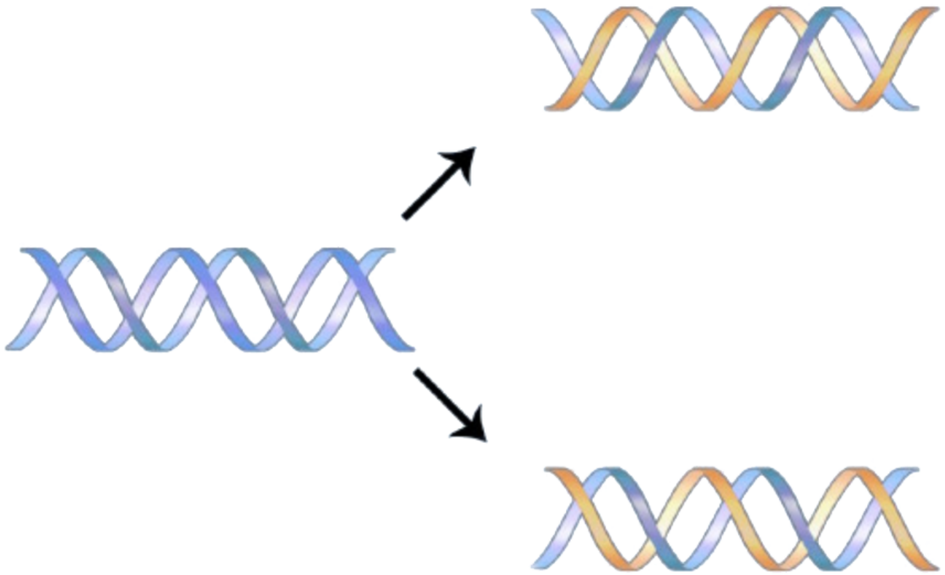
• A double-helical parental DNA molecule must be unwound to expose single strands of DNA that can serve as templates for the synthesis of new DNA strands.

• This unwinding must be accomplished without introducing significant topological distortion into the molecule.

• The unwound single strands of DNA must be kept from coming back together long enough for the new strands to be synthesized.

See Kevin's YouTube lectures on DNA Replication and Repair HERE, HERE, HERE, and HERE

- DNA polymerases cannot begin synthesis of a new DNA strand *de novo* and require a free 3' OH to which they can add DNA nucleotides.
- DNA polymerases can only extend a strand in the 5' to 3' direction. The 5' to 3' extension of both new strands at a single replication fork means that one of the strands is made in pieces.
- The use of RNA primers requires that the RNA nucleotides must be removed and replaced with DNA nucleotides and the resulting DNA fragments must be joined.
- Ensuring accuracy in the copying of so much information.


Semi-Conservative DNA Replication

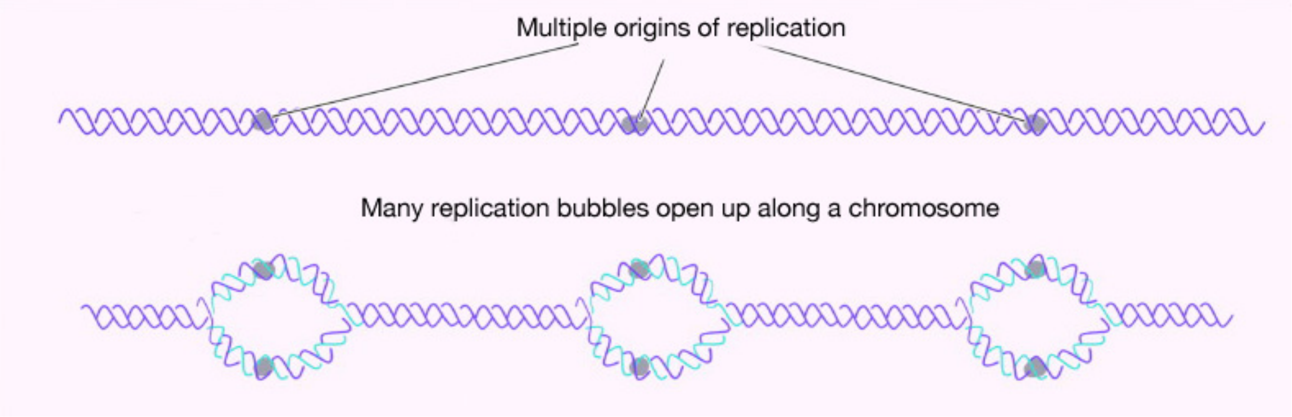
With this in mind, we can begin to examine how cells deal with each of these challenges. Our understanding of the process of DNA replication is derived from studies using bacteria, yeast, and other systems, such as *Xenopus* eggs. These investigations have revealed that DNA replication is carried out by the action of a large number of proteins that act together as a complex protein machine called the replisome. Numerous proteins involved in replication have been identified and characterized, including multiple different DNA polymerases in both prokaryotes and eukaryotes. Although the specific proteins involved are different in bacteria and eukaryotes, it is useful to understand the basic considerations that are relevant in all cells, before attempting to address the details of each system. A generalized account of the steps in DNA replication is presented below, focused on the challenges mentioned above.

- The sheer number of nucleotides to be copied is enormous.

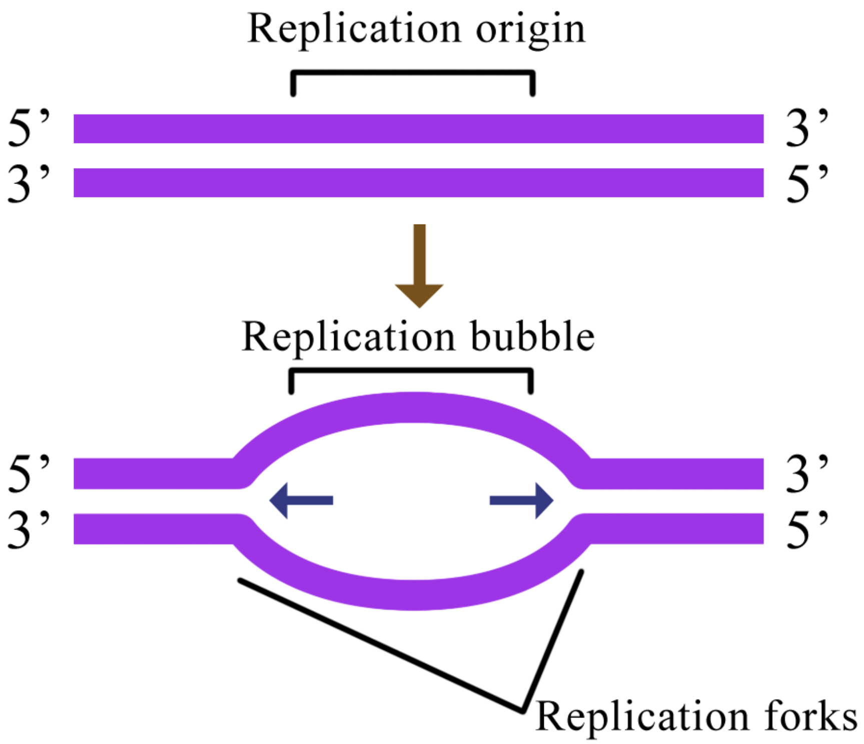
For example, in human cells, the number of nucleotides to be copied is on the order of several billion. Even in bacteria, the number is in the millions. Cells, whether bacterial or eukaryotic, have to replicate all of their DNA before they can divide. In cells like our own, the vast amount of DNA is broken up into many chromosomes, each of which is composed of a linear strand of DNA. In cells like those of *E. coli*, there is a single circular chromosome.

In either situation, DNA replication is initiated at sites called origins of replication. These are regions of the DNA molecule that are recognized by special origin recognition proteins that bind the DNA. The binding of these proteins helps open up a region of single-stranded DNA where the synthesis of new DNA can begin.

In the case of *E. coli*, there is a single origin of replication on its circular chromosome. In eukaryotic cells, there may be many thousands of origins of replication, with each chromosome having hundreds. DNA replication is thus initiated at multiple points along each chromosome in eukaryotes as shown in the figure at the right. Electron micrographs of replicating DNA from eukaryotic cells show many replication "bubbles" on a single chromosome.


Multiple origins of replication
Many replication bubbles open up along a chromosome

This makes sense in light of the large amount of DNA that there is to be copied in cells like our own, where beginning at one end of each chromosome and replicating all the way through to the other end from a single origin would simply take too long. This is despite the fact that the DNA polymerases in human cells are capable of building new DNA strands at the very respectable rate of about 50 nucleotides per second!


Replication origin
5'
3'
3'
5'
Replication bubble
5'
3'
3'
5'
Replication forks

- A double-helical parental molecule must be unwound to expose single strands of DNA that can serve as templates for the synthesis of new DNA strands.

Once a small region of the DNA is opened up at each origin of replication, the DNA helix must be unwound to allow replication to proceed. How are the strands of the parental DNA double helix separated?

The unwinding of the DNA helix requires the action of an enzyme called helicase. Helicase uses the energy released

See Kevin’s YouTube lectures on DNA Replication and Repair HERE, HERE, HERE, and HERE

when ATP is hydrolyzed to unwind the DNA helix. Note that each replication bubble is made up of two replication forks that "move" or open up, in opposite directions. At each replication fork, the parental DNA strands must be unwound to expose new sections of single-stranded template.

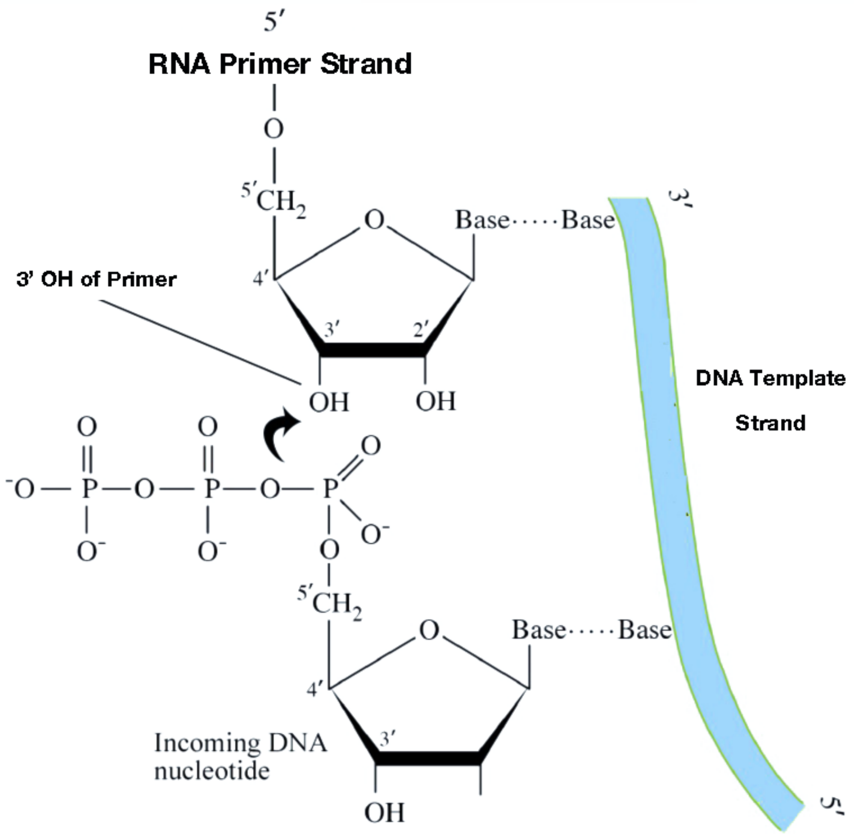
- This unwinding must be accomplished without introducing topological distortion into the molecule.

What is the effect of unwinding one region of the double helix? Unwinding the helix locally causes over-winding or topological distortion of the DNA ahead of the unwound region. The DNA ahead of the unwound helix has to rotate, or it will get twisted on itself.

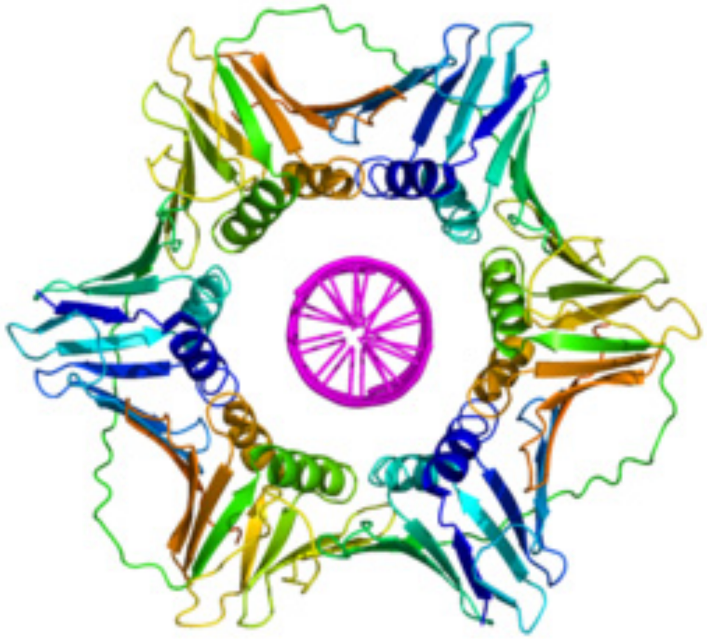
How is this problem solved? Enzymes called topoisomerases can relieve the topological stress caused by local unwinding of the double helix. They do this by cutting the DNA and allowing the strands to swivel around each other to release the tension before rejoining the ends. In *E. coli*, the topoisomerase that performs this function is called gyrase.

- The unwound single strands of DNA must be kept from coming back together long enough for the new strands to be synthesized.

Once the two strands of the parental DNA molecule are separated, they must be prevented from going back together to form double-stranded DNA. To ensure that unwound regions of the parental DNA remain single-stranded and available for copying, the separated strands of the parental DNA are bound by many molecules of a protein called single-strand DNA binding protein (SSB).



Addition of a Nucleotide to a Growing Chain



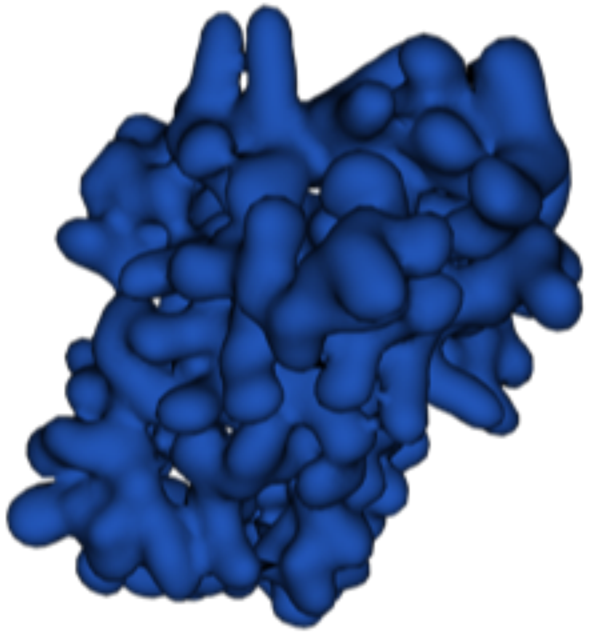
Sliding clamp assembled around DNA (in center, in purple)

- DNA polymerases cannot begin synthesis of a new DNA strand *de novo* and require a free 3' OH to which they can add DNA nucleotides.

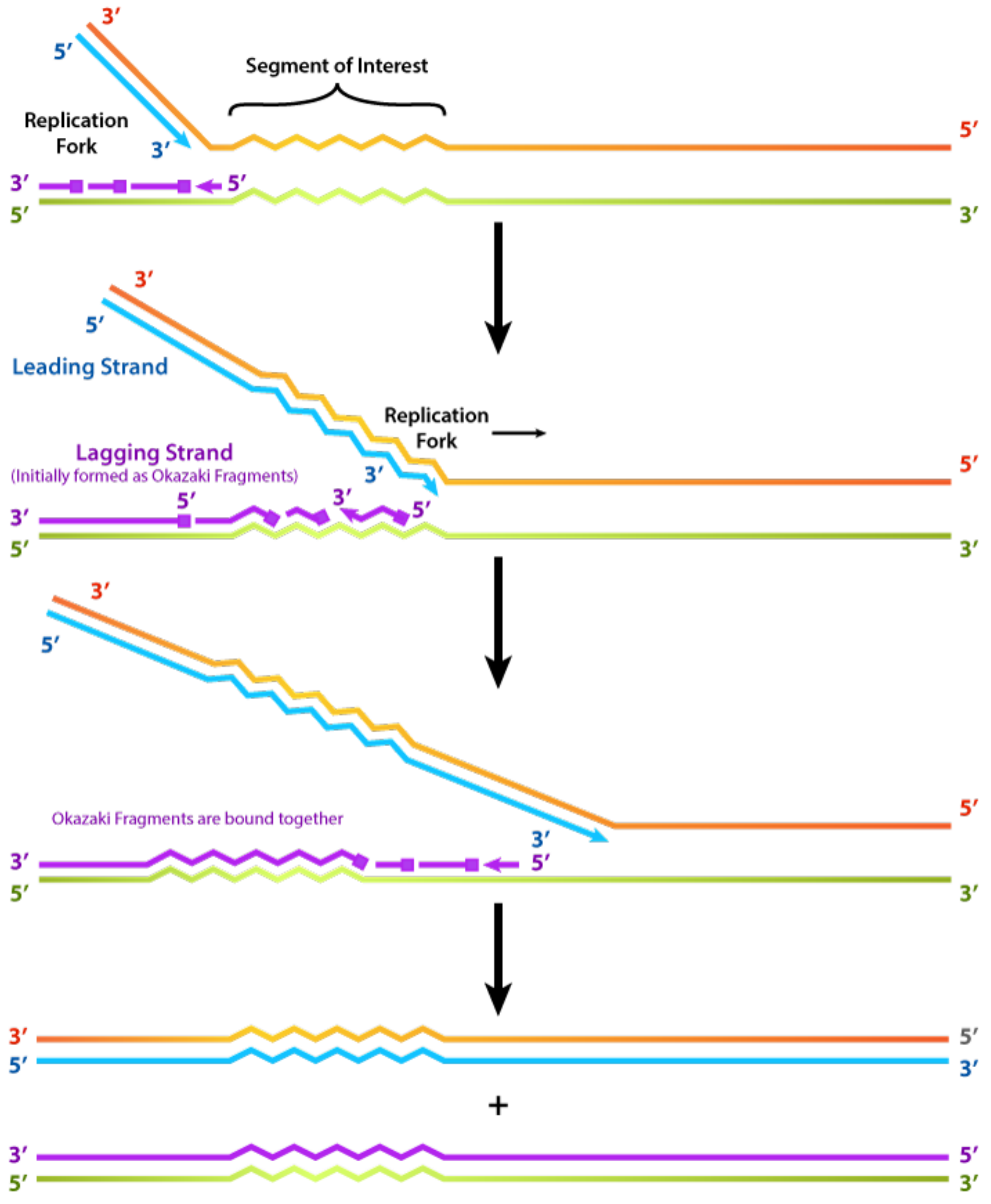
Although single-stranded parental DNA is now available for copying, DNA polymerases cannot begin synthesis of a complementary strand *de novo*. This is because all DNA polymerases can only add new nucleotides to the 3' end of a pre-existing chain. This means that some enzyme other than a DNA polymerase must first make a small region of nucleic acid, complementary to the parental strand, that can provide a free 3' OH to which DNA polymerase can add a deoxyribonucleotide.

This task is accomplished by an enzyme called a primase, which assembles a short stretch of RNA, called the primer, across from the parental DNA template. This provides a short base-paired region with a free 3'OH group to which DNA polymerase can add the first new DNA nucleotide (see figure on previous page)

Interactive 5.1



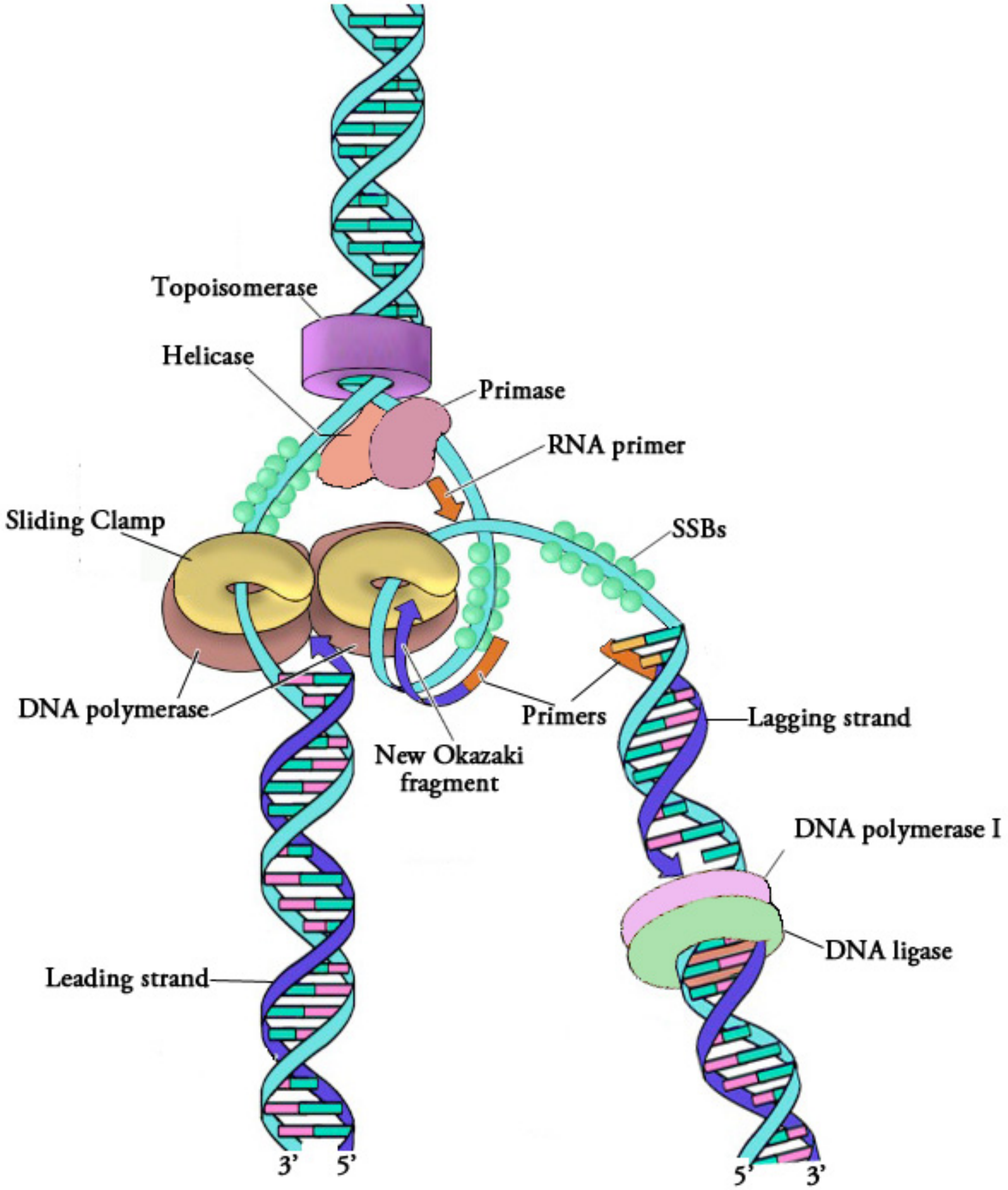
Sliding Clamp Subunit


3′
5′
Segment of Interest
Replication Fork
3′
5′
3′
5′
3′
5′
3′
3′
5′
Leading Strand
Replication Fork
Lagging Strand
(Initially formed as Okazaki Fragments)
3′
5′
3′
5′
3′
5′
5′
3′
3′
5′
Okazaki Fragments are bound together
3′
5′
3′
5′
5′
3′
3′
5′
5′
3′
+
3′
5′
5′
3′

Leading and Lagging Strand Replication

Once a primer provides a free 3'OH for extension, other proteins get into the act. These proteins are involved in loading the DNA polymerase onto the primed template and help to keep it attached to the DNA once it's on.

The first of these is the clamp loader. As its name suggests, the clamp loader helps to load a protein complex called the sliding

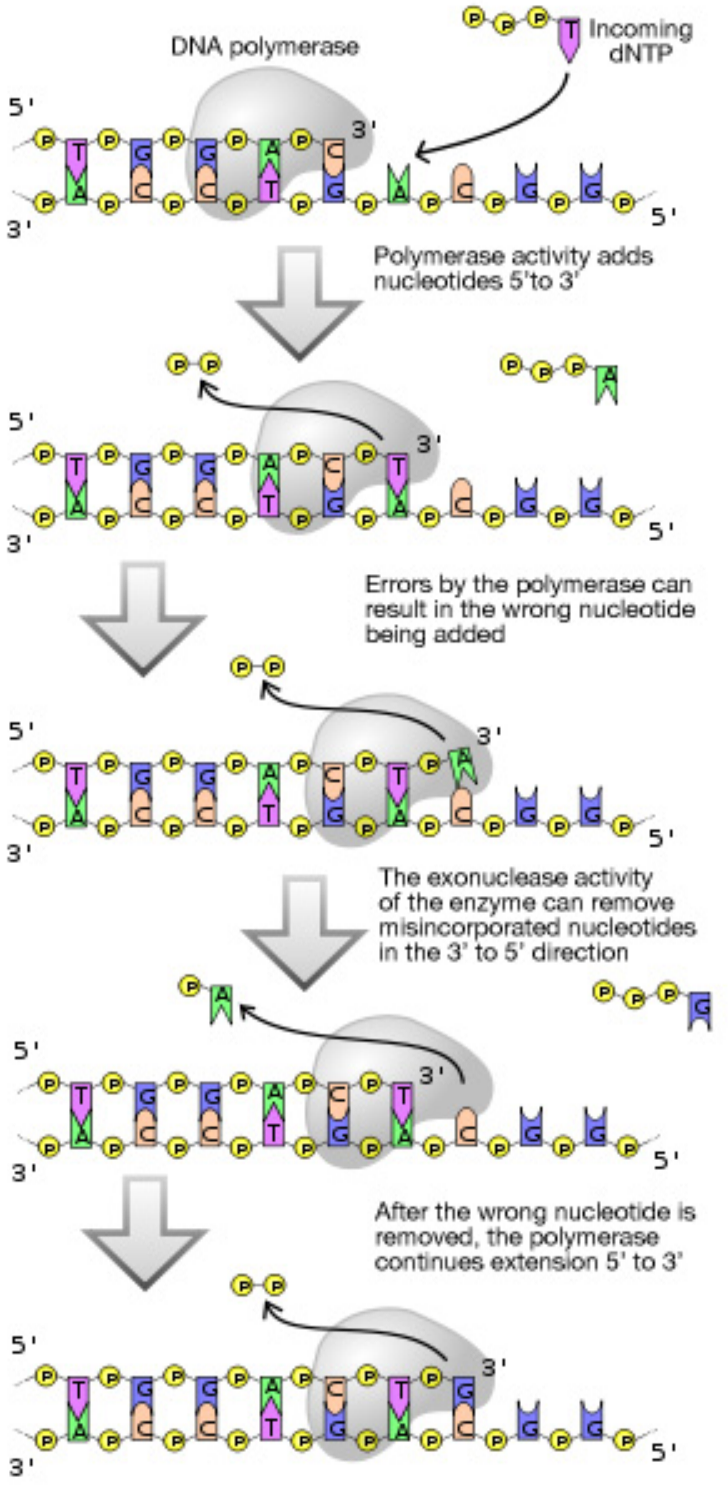

Topoisomerase
Helicase
Primase
RNA primer
Sliding Clamp
SSBs
DNA polymerase
Primers
Lagging strand
New Okazaki fragment
DNA polymerase I
DNA ligase
Leading strand
3'
5'
5'
3'

Proteins at a DNA Replication Fork

clamp onto the DNA at the replication fork. The sliding clamp is then joined by the DNA Polymerase. The function of the sliding clamp is to increase the processivity of the DNA polymerase. This is a fancy way of saying that it keeps the polymerase associated with the replication fork by preventing it from falling off- in fact, the sliding clamp has been described as a seat-belt for the DNA polymerase.

See Kevin’s YouTube lectures on DNA Replication and Repair HERE, HERE, HERE, and HERE

The DNA polymerase is now poised to start synthesis of the new DNA strand (in *E. coli*, the primary replicative polymerase is called DNA polymerase III). As you already know, the synthesis of new DNA is accomplished by the addition of new nucleotides complementary to those on the parental strand. DNA polymerase catalyzes the reaction by which an incoming deoxyribonucleotide is added onto the 3' end of the previous nucleotide, starting with the 3'OH on the end of the RNA primer.


DNA polymerase
Incoming dNTP
5'
3'
Polymerase activity adds nucleotides 5'to 3'
Errors by the polymerase can result in the wrong nucleotide being added
The exonuclease activity of the enzyme can remove misincorporated nucleotides in the 3' to 5' direction
After the wrong nucleotide is removed, the polymerase continues extension 5' to 3'

From Wikimedia Commons

The 5' phosphate on each incoming nucleotide is joined by the DNA polymerase to the 3' OH on the end of the growing nucleic acid chain. As we already noted, the new DNA strands are synthesized by the addition of DNA nucleotides to the end of an RNA primer. The new DNA molecule thus has a short piece of RNA at the beginning.

•DNA polymerases can only extend a strand in the 5' to 3' direction. The 5' to 3' growth of both new strands means that one of the strands is made in pieces.

We have noted that DNA polymerase can only build a new DNA strand in the 5' to 3' direction. We also know that the two parental strands of DNA are antiparallel. This means that at each replication fork, one new strand, called the leading strand can be synthesized continuously in the 5' to 3' direction because it is being made in the same direction that the replication fork is opening up.

The synthesis of the other new strand, called the lagging strand, requires that multiple RNA

Central Dogma Zen (part 1)

To the tune of "*Those Were the Days*"

Once upon a time a cell decided
The time was ripe for it to split in two
Had to copy cellular instructions
For the daughter cell would need them too.

Bring in a helicase
Unzip the DNAs
To ease the stress a gyrase joins the fray
Strands must be held apart
SSBs do their part
And primase builds a primer RNA.

Sliding clamp comes in behind clamp loader
dNTPs floating all around
In the wings a replicase is waiting
For the chance to start another round.

Polymerase, my friend
Starts at the 3' end
It puts a 'T' across from every 'A'
A 'G' across from 'C'
Perfect simplicity
The leading strand is made in just this way.

The lagging strand is made in little pieces
Okazaki fragments, you recall
Pol I fills the gaps that lie between them
Ligase comes in next and joins them all.

Blueprints can't have mistakes
That's why polymerase
Corrects its work with exonuclease
Proofreading one by one
Till all its work is done
Hurray for D-N-A polymerase!

Recording by Tim Karplus
Lyrics by Indira Rajagopal

primers must be laid down and the new DNA be made in many short pieces that are later joined.These short nucleic acid pieces, each composed of a small stretch of RNA primer and about 1000-2000 DNA nucleotides, are called Okazaki fragments, for Reiji Okazaki, the scientist who first demonstrated their existence.

- The use of RNA primers requires that the RNA nucleotides must be removed and replaced with DNA nucleotides.

We have seen that each newly synthesized piece of DNA starts out with an RNA primer, effectively making a new nucleic acid strand that is part RNA and part DNA. The finished DNA strand cannot be allowed to have pieces of RNA attached.

So the RNA nucleotides are removed and the gaps are filled in with DNA nucleotides (by DNA polymerase I in *E. coli*). The DNA pieces are then joined together by the enzyme DNA ligase.

The steps outlined above essentially complete the process of DNA replication. The figure on the previous page shows a replication fork, complete with the associated proteins that form the replisome.

- Ensuring accuracy in the copying of so much information

See Kevin's YouTube lectures on DNA Replication and Repair HERE, HERE, HERE, and HERE

How accurate is the copying of information in the DNA by DNA polymerase? As you are aware, changes in DNA sequence (mutations) can change the amino acid sequence of the encoded proteins and that this is often, though not always, deleterious to the functioning of the organism. When billions of bases in DNA are copied during replication, how do cells ensure that the newly synthesized DNA is a faithful copy of the original information?

DNA polymerases, as we have noted earlier, work fast (averaging 50 bases a second in human cells and up to 20 times faster in *E. coli*). Yet, both human and bacterial cells seem to replicate their DNA quite accurately. This is because of the proof-reading function of DNA polymerases. The proof-reading function of a DNA polymerase enables the polymerase to detect when the wrong base has been inserted across from a template strand, back up and remove the mistakenly inserted base. This is possible because the polymerase is a dual-function enzyme. It can extend a DNA chain by virtue of its 5' to 3' polymerase activity but it can also backtrack and remove the last inserted base because it has a 3' to 5' exonuclease activity (an exonuclease is an enzyme that removes bases, one by one, from the ends of nucleic acids). The exonuclease activity of the DNA polymerase allows it to excise a wrongly inserted base, after which the polymerase activity inserts the correct base and proceeds with extending the strand.

In other words, DNA polymerase is monitoring its own accuracy (also termed its fidelity) as it makes new DNA, correcting mistakes immediately before moving on to add the next base. This mechanism, which operates during DNA replication, corrects many errors as they occur, reducing by about 100-fold the mistakes made when DNA is copied.

Maintaining the Integrity of the Cell's Information: DNA Repair

In the last section we considered the ways in which cells deal with the challenges associated with replicating their DNA, a vital process for all cells. It is evident that if DNA is the master copy of instructions for an organism, then it is important not to make mistakes when copying the DNA to pass on to new cells. Although proofreading by DNA polymerases greatly increases the accuracy of replication, there are additional mechanisms in cells to further ensure that newly replicated DNA is a faithful copy of the original, and also to repair damage to DNA during the normal life of a cell.

All DNA suffers damage over time, from exposure to ultraviolet and other radiation, as well as from various chemicals in the environment. Even chemical reactions naturally occurring within cells can give rise to compounds that can damage DNA. As you already know, even minor changes in DNA sequence, such as point mutations can sometimes have far-reaching consequences. Likewise, unrepaired damage caused by radiation, environmental chemicals or even normal cellular chemistry can interfere with the accurate transmission of information in DNA. Maintaining the integrity of the cell's "blueprint" is of vital importance and this is reflected in the numerous mechanisms that exist to repair mistakes and damage in DNA.

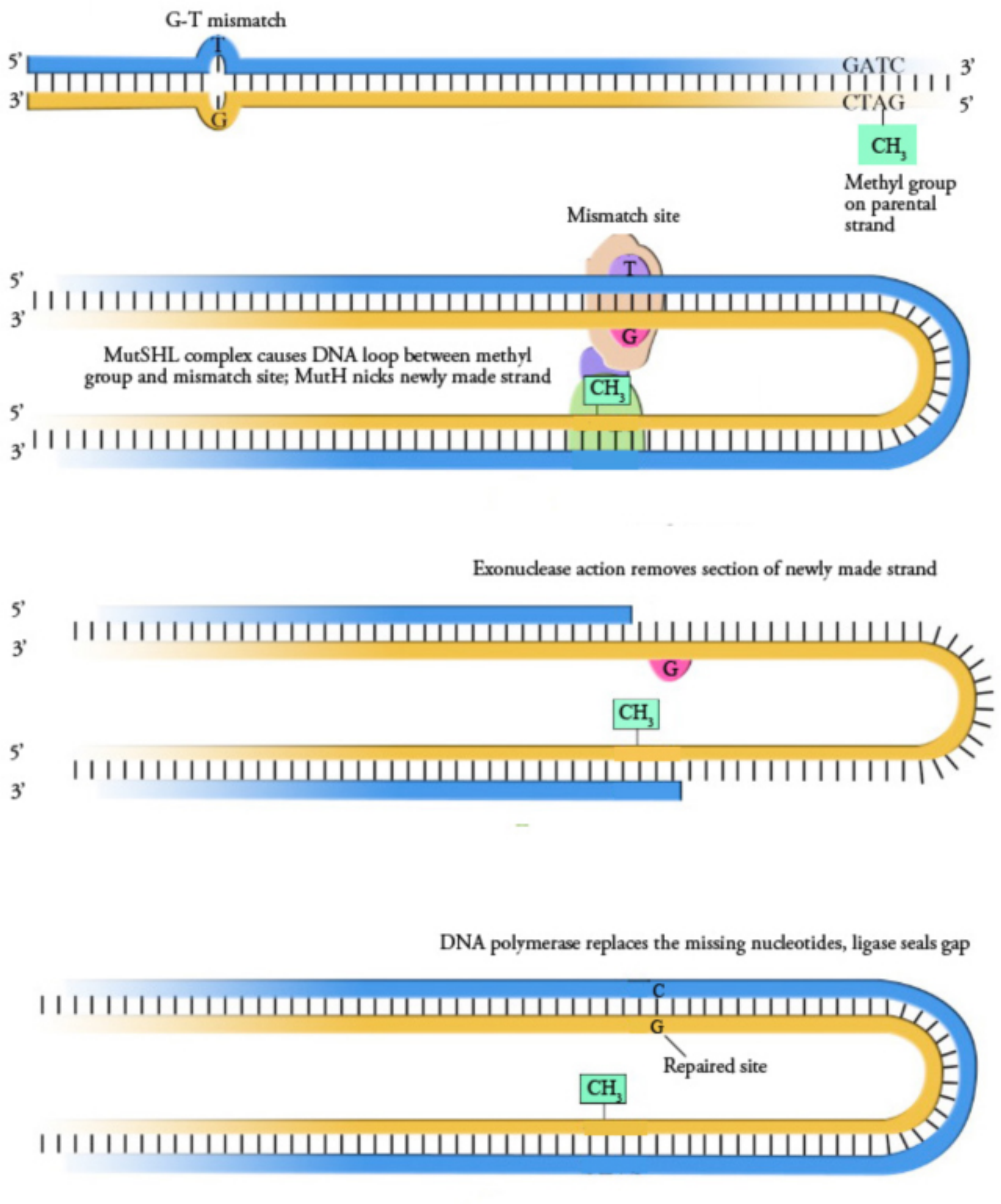
Post-Replicative Mismatch Repair

We earlier discussed proof-reading by DNA polymerases during replication. Does proofreading eliminate all errors made during replication? No. While proof-reading significantly reduces the error rate, not all mistakes are fixed on the fly by DNA polymerases. What mechanisms exist to correct the replication errors that are missed by the proof-reading function of DNA polymerases?

Errors that slip by proofreading during replication can be corrected by a mechanism called mismatch repair. While the error rate of DNA replication is about one in 10^7 nucleotides in the absence of mismatch repair, this is further reduced a hundred-fold to one in 10^9 nucleotides when mismatch repair is functional.

What are the tasks that a mismatch repair system faces?

It must:
- Scan newly made DNA to see if there are any mispaired bases (e.g., a G paired to a T)
- Identify and cut out the region of the mismatch.
- Correctly fill in the gap created by the excision of the mismatch region.


G-T mismatch
5'
3'
T
G
GATC
CTAG
3'
5'
CH_3
Methyl group on parental strand
Mismatch site
5'
3'
T
G
MutSHL complex causes DNA loop between methyl group and mismatch site; MutH nicks newly made strand
CH_3
5'
3'
Exonuclease action removes section of newly made strand
5'
3'
G
CH_3
5'
3'
DNA polymerase replaces the missing nucleotides, ligase seals gap
C
G
Repaired site
CH_3

Mismatch Repair

Importantly, the mismatch repair system must have a means to distinguish the newly made DNA strand from the template strand, if replication errors are to be fixed correctly. In other words, when the mismatch repair system encounters an A-G mispair, for example, it must know whether the A should be removed and replaced with a C or if the G should be removed and replaced with a T.

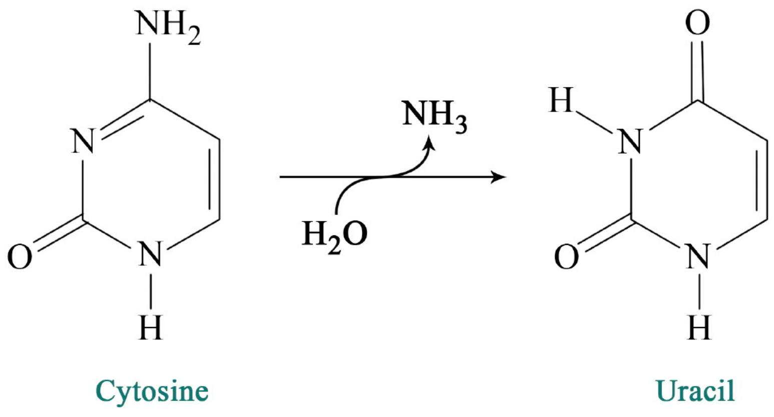
Mismatch repair has been well studied in bacteria, and the proteins involved have been identified. Eukaryotes have a mismatch repair system that repairs not only single base mismatches but also insertions and deletions.

In bacteria, mismatch repair proteins are encoded by a group of genes collectively known as the mut genes. Some of the most important components of the mismatch repair machinery are the proteins MutS, L and H.

MutS acts to recognize the mismatch, while MutL and MutH are recruited to the mismatch site by the binding of Mut S, to help cut out the region containing the mismatch. A DNA polymerase and ligase fill in the gap and join the ends, respectively.

But how does the mismatch repair system distinguish between the original and the new strands of DNA? In bacteria, the existence of a system that methylates the DNA at GATC sequences is the solution to this problem.

E.coli has an enzyme that adds methyl groups on the to adenines in GATC sequences. Newly replicated DNA lacks this methylation and thus, can be distinguished from the template strand, which is methylated. In the figure on the previous page,



Deamination of Cytosine

the template strand shown in yellow is methylated at GATC sequences.

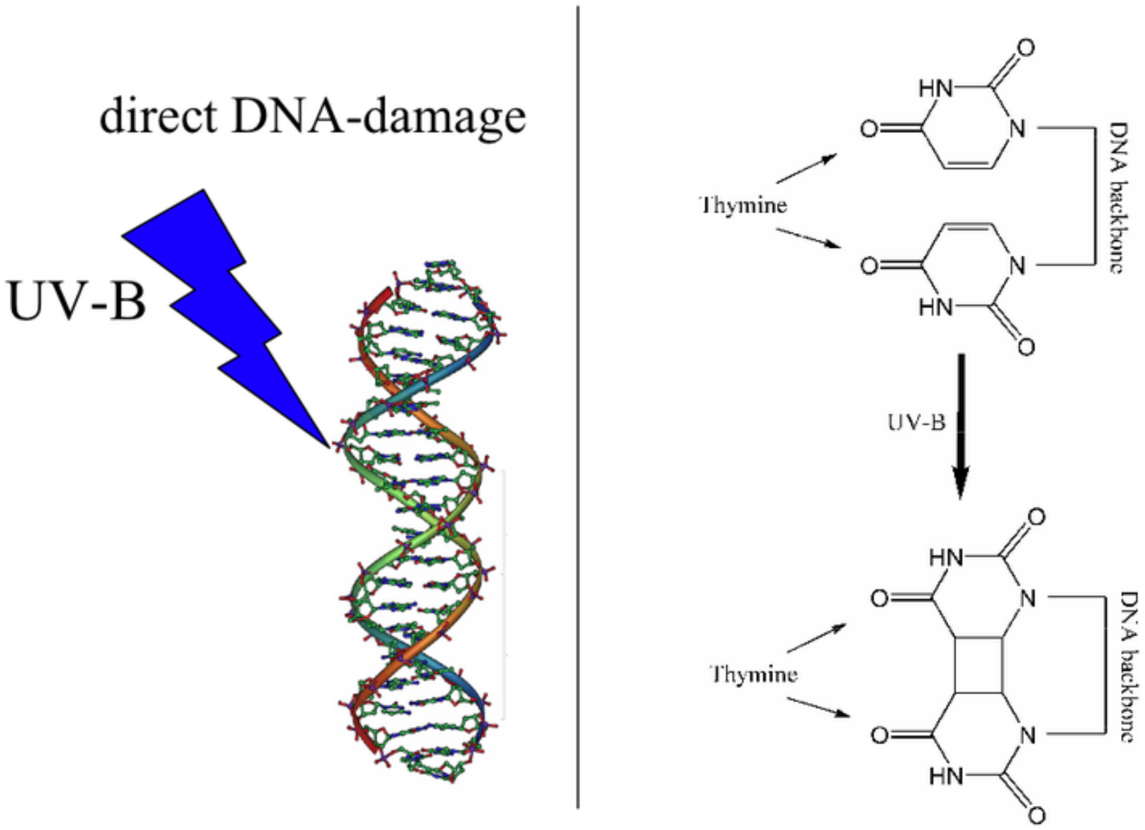
The mismatch repair proteins selectively replace the strand lacking methylation, shown in blue in the figure, thus ensuring that it is mistakes in the newly made strand that are removed and replaced. Because methylation is the criterion that enables the mismatch repair system to choose the strand that is repaired, the bacterial mismatch repair system is described as being *methyl-directed*.

Eukaryotic cells do not use this mechanism to distinguish the new strand from the template, and it is not yet understood how the mismatch repair system in eukaryotes "knows" which strand to repair.

Systems to Repair Damage to DNA

In the preceding section we discussed mistakes made when DNA is copied, where the wrong base is inserted during synthesis of the new strand. But even DNA that is not being replicated can get damaged or mutated. These sorts of damage are not associated with DNA replication, rather they can occur at any time.

What causes damage to DNA?
Some major causes of DNA damage are:
- Radiation (e.g., UV rays in sunlight, in tanning booths)
- Exposure to damaging chemicals (such as benzopyrene in car exhaust and cigarette smoke)
- Chemical reactions within the cell (such as the deamination of cytosine to give uracil).



Thymine Dimer Formation

This means the DNA in your cells is vulnerable to damage simply from normal sorts of actions, such as walking outdoors, being in traffic, or from the chemical transformations occurring in every cell as part of its everyday activities. (Naturally, the damage is much worse in situations where exposure to radiation or damaging chemicals is greater, such as when people repeatedly use tanning beds or smoke.)

What kinds of damage do these agents cause? Radiation can cause different kinds of damage to DNA. Sometimes, as with much of the damage done by UV rays, two adjacent pyrimidine bases in the DNA will be cross-linked to form pyrimidine dimers (note that we are talking about two neighboring pyrimidine bases on the same strand of DNA). This is illustrated in the figure on the previous page where two adjacent thymines on a single DNA strand are cross-linked to form a thymine dimer. Radiation can also cause breaks in the DNA backbone.

The Three R's of DNA

To the tune of "*Dream of Little Dream of Me*"

Base pairs they all provide you
Stair steps to form a helix inside you
A pairs with T and G goes with C
Making DNA for me

Helicases go unwinding
Unzippering at rates almost blindin'
PO-lymerases work night and day
Replicating D-N-A

(Bridge)
Proof-reading - the enzyme's QC path
Chews back from the 3's
I can't have a 'G' paired with 'T' so
Repair it please

Chem damage is concern too
'Cuz it can cause mutations inside you
When dimers stem from sunlight UV
Fix the DNA for me

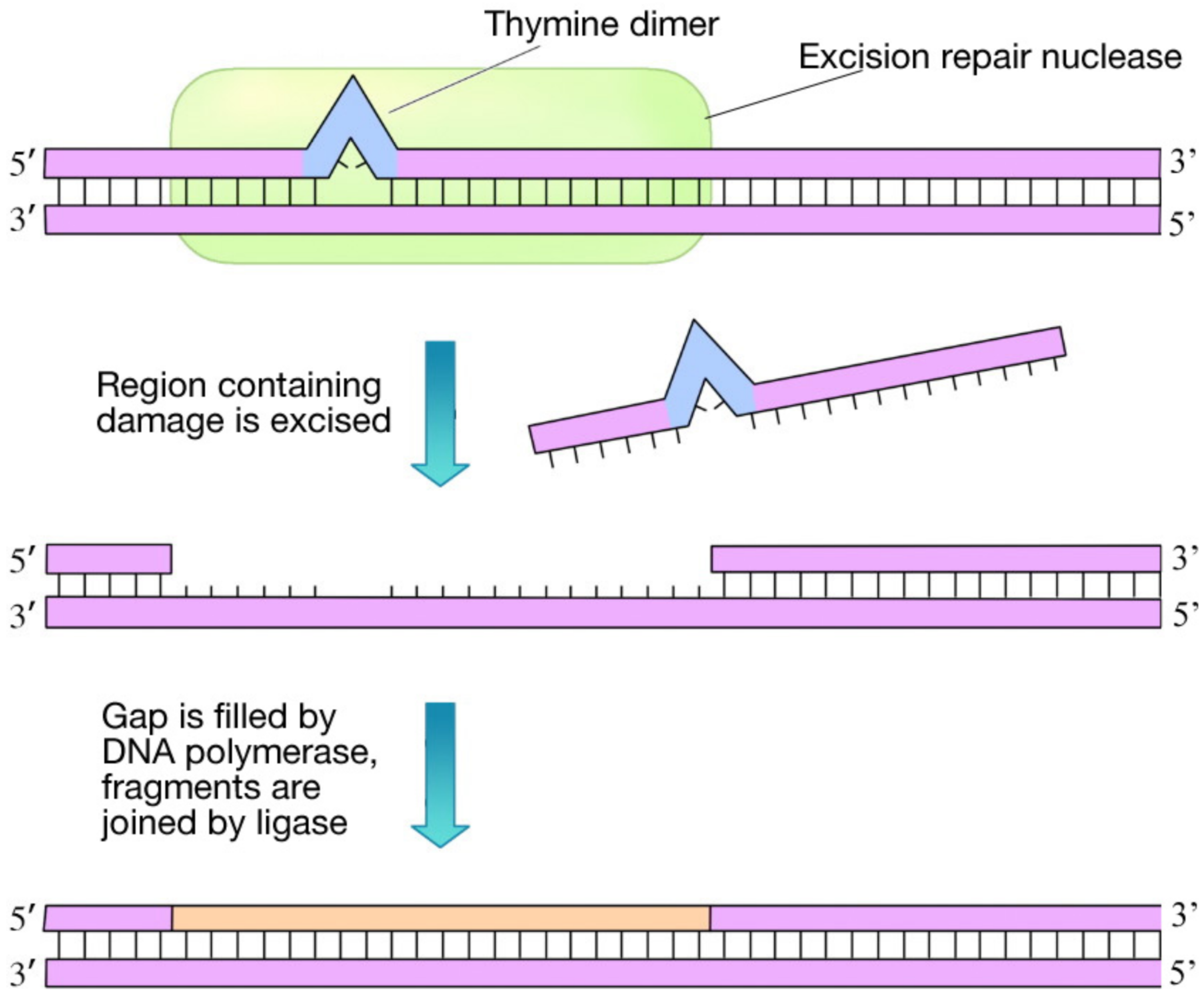
Such pathways of excision
Cause cells to have to make a decision
Should they go straight ahead with repair
Or take themselves right out of there?

(Bridge)
Then lastly, there's recombination
Swap strands readily
Crossover homologous regions
Mix them for me

This story is complete now
The DNA is fit for gametes now
The three R's for the DNA shine
Replicate, repair, recombine

(Oh yeah!)
Replicate, repair, recombine

Recorded by Liz Bacon and David Simmons
Lyrics by Kevin Ahern


Thymine dimer
Excision repair nuclease
5′
3′
3’
5’
Region containing damage is excised
Gap is filled by DNA polymerase, fragments are joined by ligase

Thymine Dimer Removal

Chemicals like benzopyrene can attach themselves to bases, forming bulky DNA adducts in which large chemical groups are linked to bases in the DNA. The formation of chemical adducts can physically distort the DNA helix, making it hard for DNA and RNA polymerases to copy those regions of DNA.

Chemical reactions occurring within cells can cause cytosines in DNA to be deaminated to uracil, as shown in the figure above.

Other sorts of damage in this category include the formation of oxidized bases like 8-oxo-guanine. These do not actually change the physical structure of the DNA helix, but they can cause problems because uracil and 8-oxo-guanine pair with different bases than the original cytosine or guanine, leading to mutations on the next round of replication.

How do cells repair such damage? Cells have several ways to remove the sorts of damage described above, with excision repair being a common strategy. Excision repair is a general term for the cutting out and re-synthesis of the damaged region of the DNA. There are a couple of varieties of excision repair:

Nucleotide Excision Repair (NER)

This system fixes damage by chemicals as well as UV damage. As shown in the figure on the previous page, in nucleotide excision repair, the damage is recognized and a cut is made on either side of the damaged region by an enzyme called an excinuclease (shown in green). A short portion of the DNA strand containing the damage is then removed and a DNA polymerase fills in the gap with the appropriate nucleotides. The newly made DNA is joined to the rest of the DNA backbone by the enzyme DNA ligase. In *E. coli*, NER is carried out by a group of proteins encoded by the uvrABC genes. As you can see, NER is similar, in principle, to mismatch repair. However, in NER, the distortion of the helix, caused by the DNA damage, clearly indicates which strand of the DNA needs to be removed and replaced.

Base Excision Repair (BER)

BER deals with situations like the deamination of cytosine to uracil. As noted earlier, cytosines in DNA sometimes undergo deamination to form the base uracil.

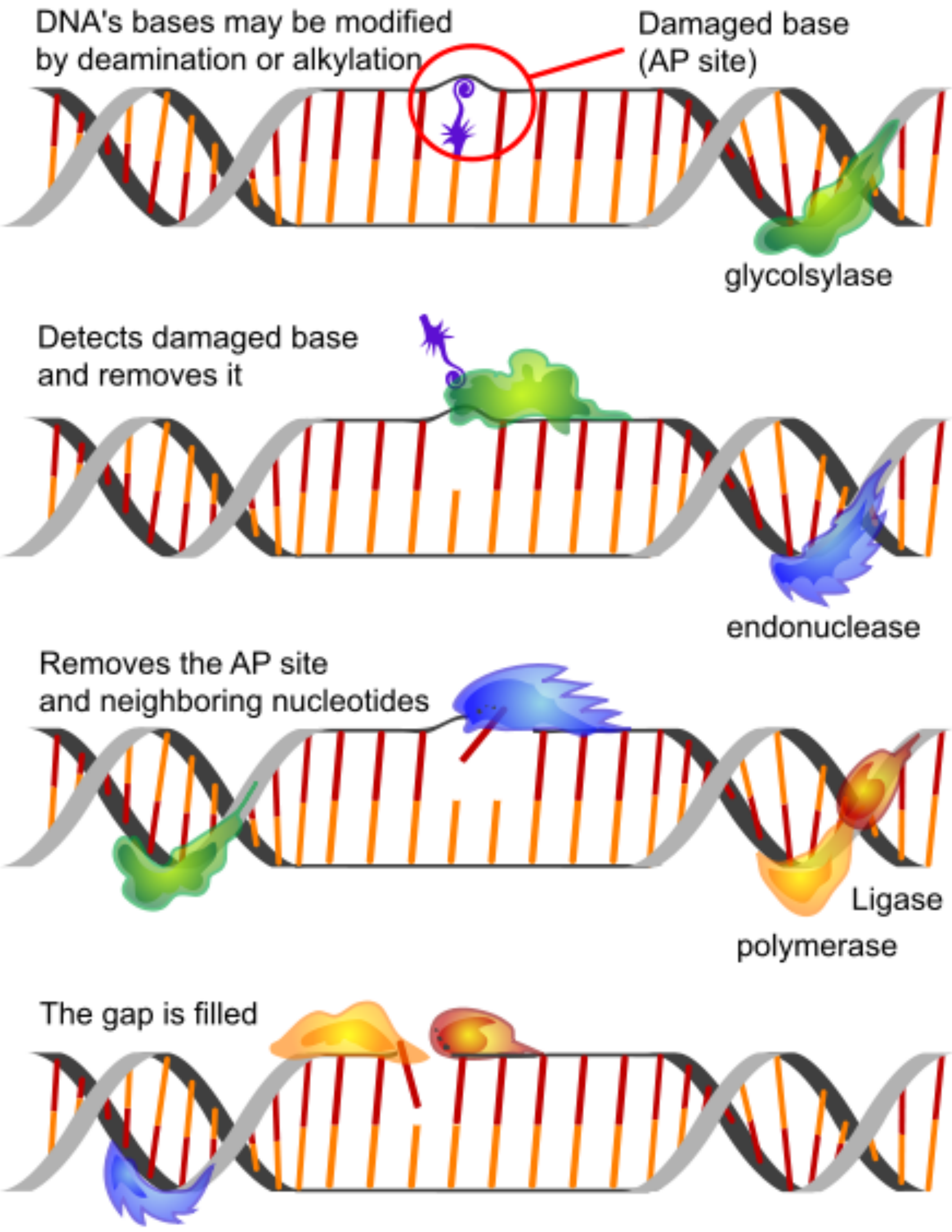
Because cytosines pair with guanines and uracils pair with adenine, the conversion of cytosine to uracil in the DNA would lead to the insertion of an A in the newly replicated strand instead of the G that should have gone in across from a C. To prevent this from happening, uracils are removed from DNA by base excision repair.

In base excision repair, a single base is first removed from the DNA, followed by removal of a region of the DNA surrounding the missing base. The gap is then repaired.

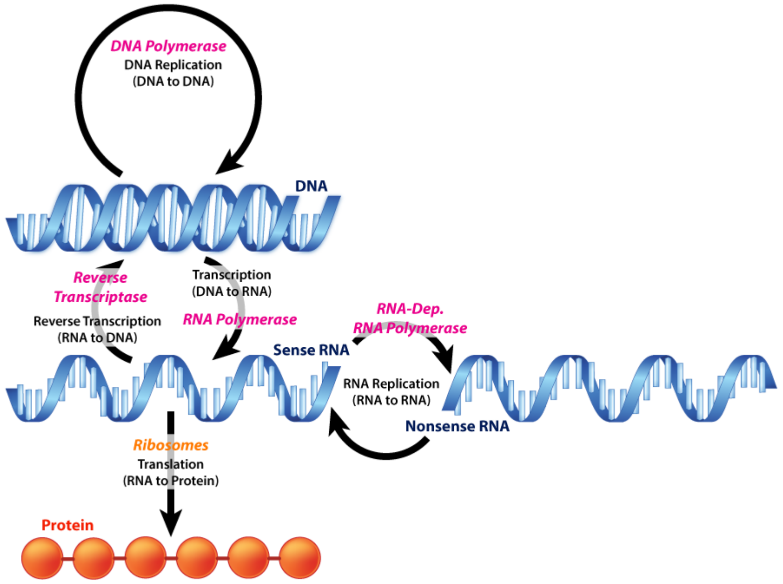
The removal of uracil from DNA is accomplished by the enzyme uracil DNA glycosylase, which breaks the bond between the uracil and the sugar in the nucleotide.

The removal of the uracil base creates a gap called an apyrimidinic site (AP site). The presence of the AP site triggers the activity of an AP endonuclease that cuts the DNA backbone.

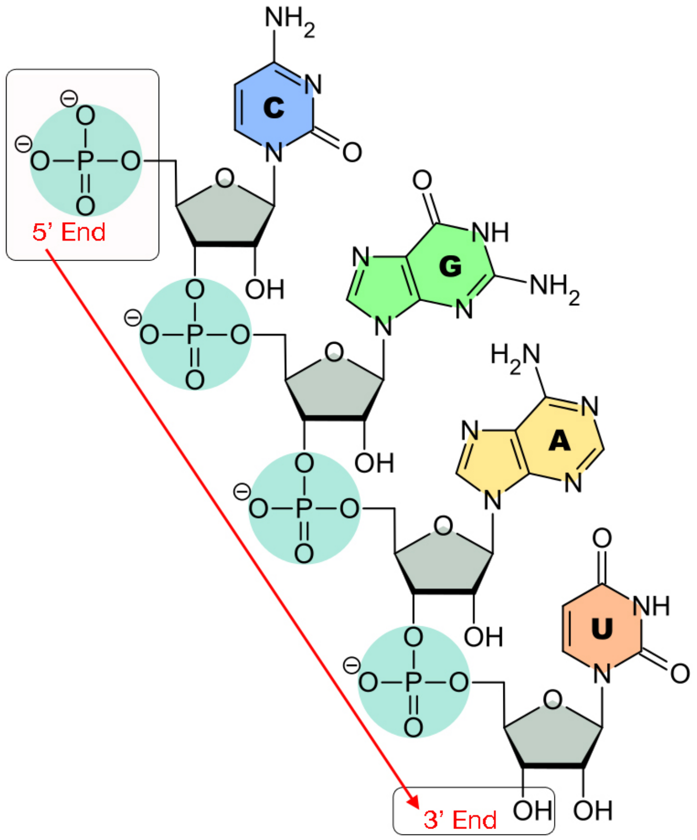
A short region of the DNA surrounding the site of the original uracil is then removed and replaced.


DNA's bases may be modified
by deamination or alkylation
Damaged base
(AP site)
glycolsylase
Detects damaged base
and removes it
endonuclease
Removes the AP site
and neighboring nucleotides
Ligase
polymerase
The gap is filled

Base Excision Repair


DNA Polymerase
DNA Replication
(DNA to DNA)
DNA
Reverse Transcriptase
Reverse Transcription
(RNA to DNA)
Transcription
(DNA to RNA)
RNA Polymerase
RNA-Dep. RNA Polymerase
Sense RNA
RNA Replication
(RNA to RNA)
Nonsense RNA
Ribosomes
Translation
(RNA to Protein)
Protein

Central Dogma


5' End
3' End
C
G
A
U
NH_2
H_2N
OH

RNA Structure

Transcription

In the preceding sections, we have discussed the replication of the cell's DNA and the mechanisms by which the integrity of the genetic information is carefully maintained. What do cells do with this information? How does the sequence in DNA control what happens in a cell? If DNA is a giant instruction book containing all of the cell's "knowledge" that is copied and passed down from generation to generation, what are the instructions for? And how do cells use these instructions to make what they need?

Dogma, Central

There's a dogma that's central to cells
That says that the DNA tells
The RNAs to
Make proteins for you
And they'd better get moving, or else.

You have learned in introductory biology courses that genes, which are instructions for making proteins, are made of DNA. You also know that information in genes is copied into temporary instructions called messenger RNAs that direct the synthesis of specific proteins. This description of flow of information from DNA to RNA to protein, shown on the previous page, is often called the Central Dogma of molecular biology and is a good starting point for an examination of how cells use the information in DNA.

Watch Kevin's YouTube lectures on Transcription HERE, HERE, and HERE

Consider that all of the cells in a multicellular organism have

General Features of Transcription

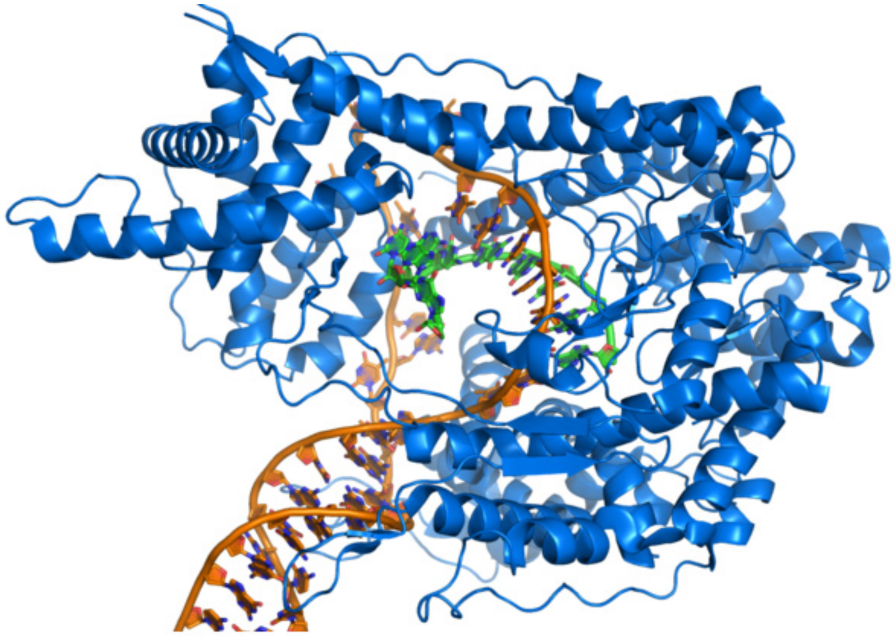
- The process of making RNA copies using a DNA as template is known as transcription
- The process of transcription produces all sorts of RNAs (mRNA, tRNA, rRNA, etc)
- One strand of the DNA serves as a template for the synthesis of RNA
- Enzymes that synthesize RNA are called RNA polymerases
- RNA polymerases synthesize RNA in the 5’ to 3’ direction
- RNA polymerases do not need a primer
- RNA polymerases uses rNTPs (ATP,GTP,UTP and CTP) to build the new RNA strand
- RNA polymerases bind at specific DNA sequences called promoters to start transcription
- RNA polymerases stop RNA synthesis when they reach sequences called terminators

arisen by division from a single fertilized egg and therefore, all have the same DNA. Division of that original fertilized egg produces, in the case of humans, over a trillion cells, by the time a baby is produced from that egg (that's a lot of DNA replication!). Yet, we also know that a baby is not a giant ball of a trillion identical cells, but has the many different kinds of cells that make up tissues like skin and muscle and bone and nerves. How did cells that have identical DNA turn out so different?

The answer lies in gene expression, which is the process by which the information in DNA is used. Although all the cells in a baby have the same DNA, each different cell type uses a different subset of the genes in that DNA to direct the synthesis of a distinctive set of RNAs and proteins. The first step in gene expression is transcription, which we will examine next.

What is transcription? Transcription is the process of copying information from DNA sequences into RNA sequences.
This process is also known as DNA-dependent RNA synthesis. When a sequence of DNA is transcribed, only one of the two DNA strands is copied into RNA.

But, apart from copying one, rather than both strands of DNA, how is transcription different from replication of DNA? DNA replication serves to copy all the genetic material of the cell and occurs before a cell divides, so that a full copy of the cell's genetic information can be passed on to the daughter cell. Transcription, by contrast, copies short stretches of the coding regions of DNA to make RNA. Different genes may be copied into RNA at different times in the cell's life cycle. RNAs are, so to speak, temporary copies of instructions of the information in DNA and different sets of instructions are copied for use at different times.



T7 RNA Polymerase making RNA (green) using DNA template (brown)

From Wikimedia Commons

Cells make several different kinds of RNA:

- mRNAs that code for proteins
- rRNAS that form part of ribosomes
- tRNAs that serve as adaptors between mRNA and amino acids during translation
- Micro RNAs that regulate gene expression
- Other small RNAs that have a variety of functions.

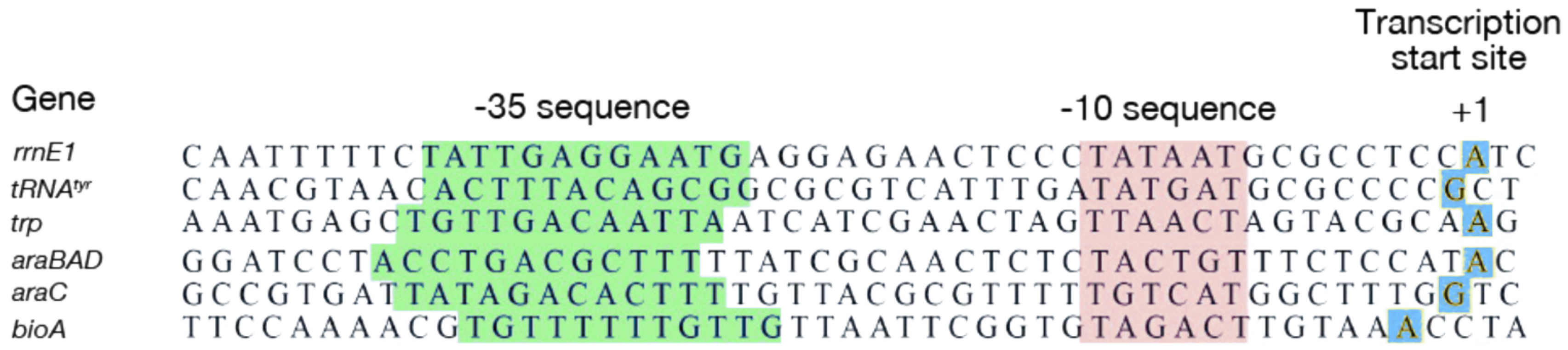
Building an RNA strand is very similar to building a DNA strand. This is not surprising, knowing that DNA and RNA are very similar molecules. What enzyme carries out transcription? Transcription is catalyzed by the enzyme RNA Polymerase. "RNA polymerase" is a general term for an enzyme that makes RNA. There are many different RNA polymerases.

Like DNA polymerases, RNA polymerases synthesize new strands only in the 5' to 3' direction, but because they are making RNA, they use ribonucleotides (i.e., RNA nucleotides) rather than deoxyribonucleotides. Ribonucleotides are joined in exactly the same way as deoxyribonucleotides, which is to say that the 3'OH of the last nucleotide on the growing chain is joined to the 5' phosphate on the incoming nucleotide.

One important difference between DNA polymerases and RNA polymerases is that the latter do not require a primer to start making RNA. Once RNA polymerases are in the right place to start copying DNA, they just begin making RNA by stringing together RNA nucleotides complementary to the DNA template.

This, of course, brings us to an obvious question- how do RNA polymerases "know" where to start copying on the DNA? Unlike the situation in replication, where every nucleotide of the parental DNA must eventually be copied, transcription, as we have already noted, only copies selected genes into RNA at any given time.

Consider the challenge here: in a human cell, there are approximately 6 billion basepairs of DNA. Most of this is non-coding DNA, meaning that it won't need to be transcribed. The small percentage of the genome that is made up of coding sequences still amounts to between 20,000 and 30,000 genes in


Transcription start site
Gene
-35 sequence
-10 sequence
+1
rrnE1
tRNA^{tyr}
trp
araBAD
araC
bioA
CAATTTTTCTATTGAGGAATGAGGAGAACTCCCTATAATGCGCCTCCATC
CAACGTAACACTTTACAGCGGCGCGTCATTTGATATGATGCGCCCCGCT
AAATGAGCTGTTGACAATTAATCATCGAACTAGTTAACTAGTACGCAAG
GGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATAC
GCCGTGATTATAGACACTTTTGTTACGCGTTTTTGTCATGGCTTTGGTC
TTCCAAAACGTGTTTTTTTGTTGTTAATTCGGTGTAGACTTGTAAACCTA

Promoter Sequences

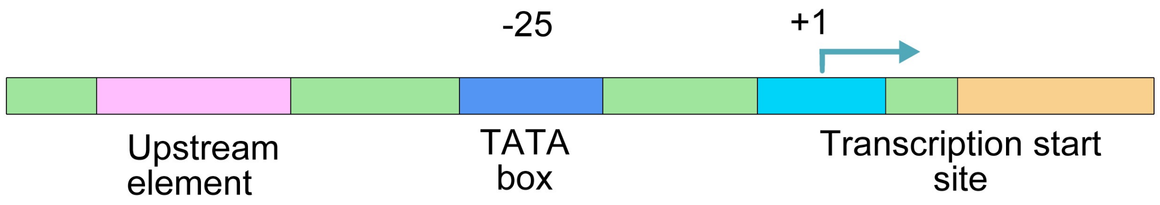
each cell. Of these genes, only a small number will need to be expressed at any given time.

What indicates to an RNA polymerase where to start copying DNA to make a transcript? Signals in DNA indicate to RNA polymerase where it should start (and end) transcription. These signals are special sequences in DNA that are recognized by the RNA polymerase or by proteins that help RNA polymerase determine where it should bind the DNA to start transcription. A DNA sequence at which the RNA polymerase binds to start transcription is called a promoter.

A promoter is generally situated upstream of the gene that it controls. What this means is that on the DNA strand that the gene is on, the promoter sequence is "before" the gene. Remember that, by convention, DNA sequences are read from 5' to 3'. So the promoter lies 5' to the start point of transcription.

Also notice that the promoter is said to "control" the gene it is associated with. This is because expression of the gene is dependent on the binding of RNA polymerase to the promoter sequence to begin transcription. If the RNA polymerase and its helper proteins do not bind the promoter, the gene cannot be transcribed and it will therefore, not be expressed.

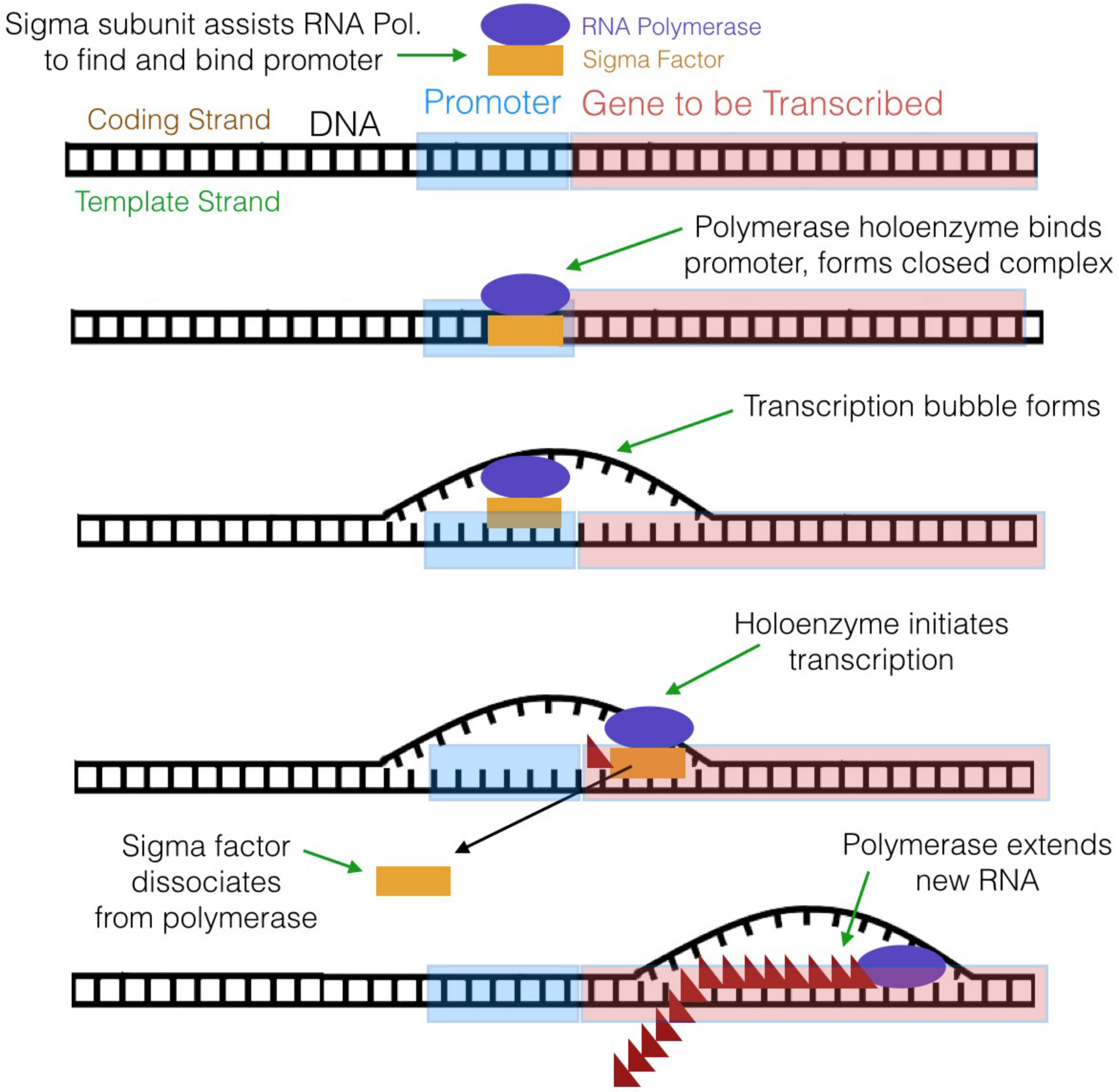
What is special about a promoter sequence? In an effort to answer this question, scientists looked at many genes and their surrounding sequences. It makes sense that because the same


-25
+1
Upstream element
TATA box
Transcription start site

Promoter Sequence Elements

RNA polymerase has to bind to many different promoters, the promoters should have some similarities in their sequences. Sure enough, common sequence patterns were seen to be present in many promoters. We will first take a look at prokaryotic promoters. When prokaryotic genes were examined, the following features commonly emerged (see figure above):

- A transcription start site (this the base in the DNA across from which the first RNA nucleotide is paired).
- A -10 sequence: this is a 6 bp region centered about 10 bp upstream of the start site. The consensus sequence at this position is TATAAT. In other words, if you count back from the transcription start site, which by convention, is called the +1, the sequence found at -10 in the majority of promoters studied is TATAAT).
- A -35 sequence: this is a sequence at about 35 basepairs upstream from the start of transcription. The consensus sequence at this position is TTGACA.


Sigma subunit assists RNA Pol. to find and bind promoter
RNA Polymerase
Sigma Factor
Coding Strand
DNA
Promoter
Gene to be Transcribed
Template Strand
Polymerase holoenzyme binds promoter, forms closed complex
Transcription bubble forms
Holoenzyme initiates transcription
Sigma factor dissociates from polymerase
Polymerase extends new RNA

Transcription Initiation in E. coli

What is the significance of these sequences? It turns out that the sequences at -10 and -35 are recognized and bound by a subunit of prokaryotic RNA polymerase before transcription can begin.

The RNA polymerase of *E. coli*, for example, has a subunit called the sigma subunit (or sigma factor) in addition to the core polymerase, which is the part of the enzyme that actually makes RNA. Together, the sigma subunit and core polymerase make up what is termed the RNA polymerase holoenzyme. The sigma subunit

Watch Kevin's YouTube lectures on Transcription HERE, HERE, and HERE of the polymerase (shown in brown in the figure) can recognize and bind to the -10 and -35 sequences in the promoter, thus positioning the RNA polymerase (shown in green) at the right place to initiate transcription. Once transcription begins, the core polymerase and the sigma subunit separate, with the core polymerase continuing RNA synthesis and the sigma subunit wandering off to escort another core polymerase molecule to a promoter. The sigma subunit can be thought of as a sort of usher that leads the polymerase to its "seat" on the promoter.

The Student’s Guide to a Perfect Transcript

Pol II's so smart, now let me see
It makes a transcript, one, two, three,
From a billion base pairs can it find
The one promoter it must bind?

I need some help, I hear it plead,
This DNA I cannot read
I cannot see where I must start
'Til TFIIs have done their part.

When TATA's bound by TBP
That sends a signal out, you see
Once DNA has made a bend
More TFIIs will soon attend.

When B arrives, it clears a place
For RNA polymerase
And F and E soon join the fun
TFIIH, the final one.

The last one is a special case
It moonlights as a helicase
And sends Pol II upon its way
To make a brand new RNA.

How does it do that, you may ask
A phosphate does the crucial task
Added on to CTD,
That's how the H sets Pol II free.

And Pol II goes its merry way
There soon will be some RNA
Then introns must be all excised
As RNA is capped and spliced.

Then PolyA polymerase
Will add a couple hundred As
For my own transcript, I can say
That all I want is one more A.

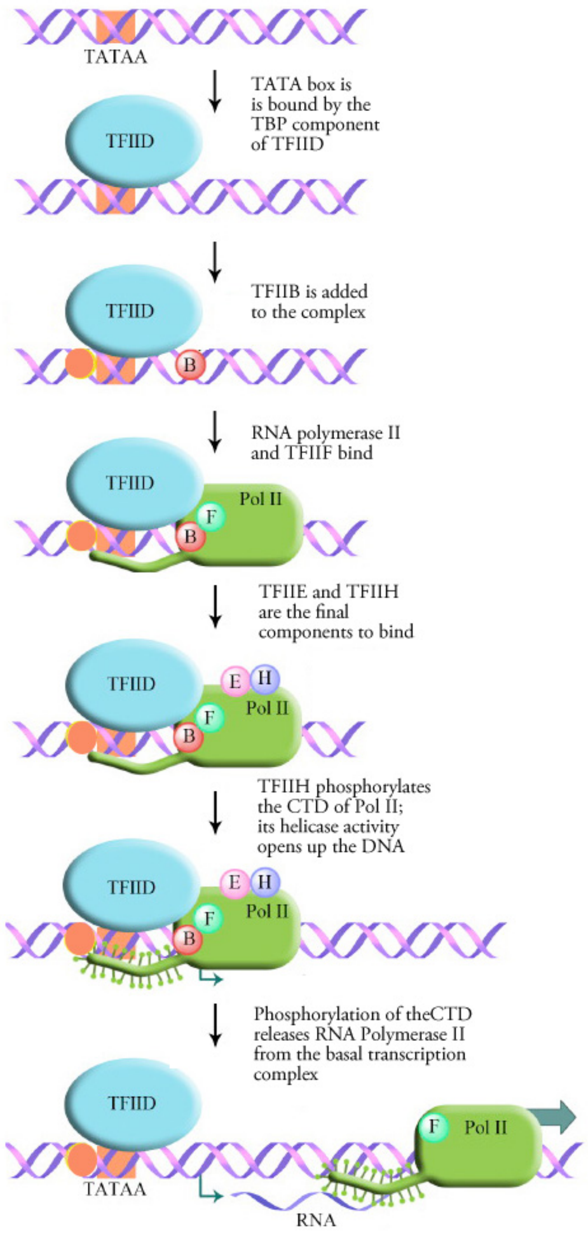
Verse by Indira Rajagopal

As already mentioned, an RNA chain, complementary to the DNA template, is built by the RNA polymerase by the joining of the 5' phosphate of an incoming ribonucleotide to the 3'OH on the last nucleotide of the growing RNA strand. How does the polymerase know where to stop? A sequence of nucleotides called the terminator is the signal to the RNA polymerase to stop transcription and dissociate from the template.

Although the process of RNA synthesis is the same in eukaryotes as in prokaryotes, there are some additional issues to keep in mind in eukaryotes. One is that in eukaryotes, the DNA template exists as chromatin, where the DNA is tightly associated with histones and other proteins. The "packaging" of the DNA must therefore be opened up to allow the RNA polymerase access to the template in the region to be transcribed.

A second difference is that eukaryotes have multiple RNA polymerases, not one as in bacterial cells. The different polymerases transcribe different genes. For example, RNA polymerase I transcribes the ribosomal RNA genes, while RNA polymerase III copies tRNA genes. The RNA polymerase we will focus on most is RNA polymerase II, which transcribes protein-coding genes to make mRNAs.

All three eukaryotic RNA polymerases need additional proteins to help them get transcription started. In prokaryotes, RNA polymerase by itself can initiate transcription (remember that the sigma subunit is a subunit of the prokaryotic RNA polymerase). The additional proteins needed by eukaryotic RNA polymerases are referred to as transcription factors. We will see below that there are various categories of transcription factors.


TATAA
TATA box is is bound by the TBP component of TFIID
TFIID
TFIIB is added to the complex
TFIID
B
RNA polymerase II and TFIIF bind
TFIID
Pol II
F
B
TFIIE and TFIIH are the final components to bind
TFIID
E
H
Pol II
F
B
TFIIH phosphorylates the CTD of Pol II; its helicase activity opens up the DNA
TFIID
E
H
Pol II
F
B
Phosphorylation of theCTD releases RNA Polymerase II from the basal transcription complex
TFIID
F
Pol II
TATAA
RNA

Assembly of the Basal Transcription Complex and Initiation of Transcription

Finally, in eukaryotic cells, transcription is separated in space and time from translation. Transcription happens in the nucleus, and the mRNAs produced are processed further before they are sent into the cytoplasm. Protein synthesis (translation) happens in the cytoplasm. In prokaryotic cells, mRNAs can be translated as they are coming off the DNA template, and because there is no nucleus, transcription and protein synthesis occur in a single cellular compartment.

Like genes in prokaryotes, eukaryotic genes also have promoters. Eukaryotic promoters commonly have a TATA box, a sequence about 25 basepairs upstream of the start of transcription that is recognized and bound by

Watch Kevin's YouTube lectures on Transcription HERE, HERE, and HERE

proteins that help the RNA polymerase to position itself correctly to begin transcription. (Some eukaryotic promoters lack TATA boxes, and have, instead, other recognition sequences to help the RNA polymerase find the spot on the DNA where it spot on the DNA where it binds and initiates transcription.)

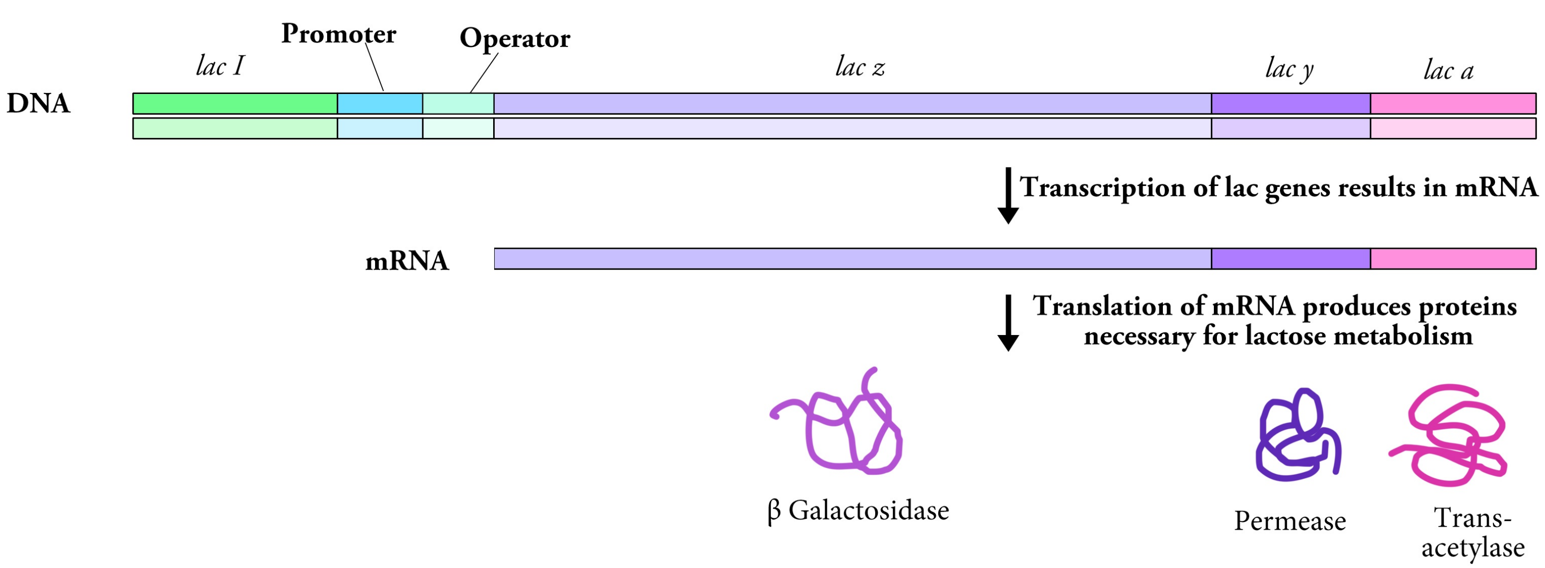
We noted earlier that eukaryotic RNA polymerases need additional proteins to bind promoters and start transcription. What are these additional proteins that are needed to start transcription? General transcription factors are proteins that help eukaryotic RNA polymerases find transcription start sites and initiate RNA synthesis. We will focus on the transcription factors that assist RNA polymerase II. These transcription factors are named TFIIA, TFIIB and so on (TF= transcription factor, II=RNA polymerase II, and the letters distinguish individual transcription factors).

Transcription in eukaryotes requires the general transcription factors and the RNA polymerase to form a complex at the TATA box called the basal transcription complex or transcription initiation complex. This is the minimum requirement for any gene to be transcribed. The first step in the formation of this complex is the binding of the TATA box by a transcription factor called the TATA Binding Protein or TBP. Binding of the TBP causes the DNA to bend at this spot and take on a structure that is suitable for the binding of additional transcription factors and RNA polymerase. As shown in the figure at left, a number of different general transcription factors, together with RNA polymerase (Pol II) form a complex at the TATA box.

The final step in the assembly of the basal transcription complex is the binding of a general transcription factor called TFIIH. TFIIH is a multifunctional protein that has helicase activity (i.e., it is capable of opening up a DNA double helix) as well as kinase activity. The kinase activity of TFIIH adds a phosphate onto the C-terminal domain (CTD) of the RNA polymerase. This phosphorylation appears to be the signal that releases the RNA polymerase from the basal transcription complex and allows it to move forward and begin transcription.

Regulation of Transcription

The processes described above are required whenever any gene is transcribed. But what determines which genes are transcribed at a given time? What are the molecular switches that turn transcription on or off? Although there are entire books written on this one topic, the basic mechanism by which transcription is regulated depends on highly specific interactions between transcription regulating proteins and regulatory sequences on DNA.

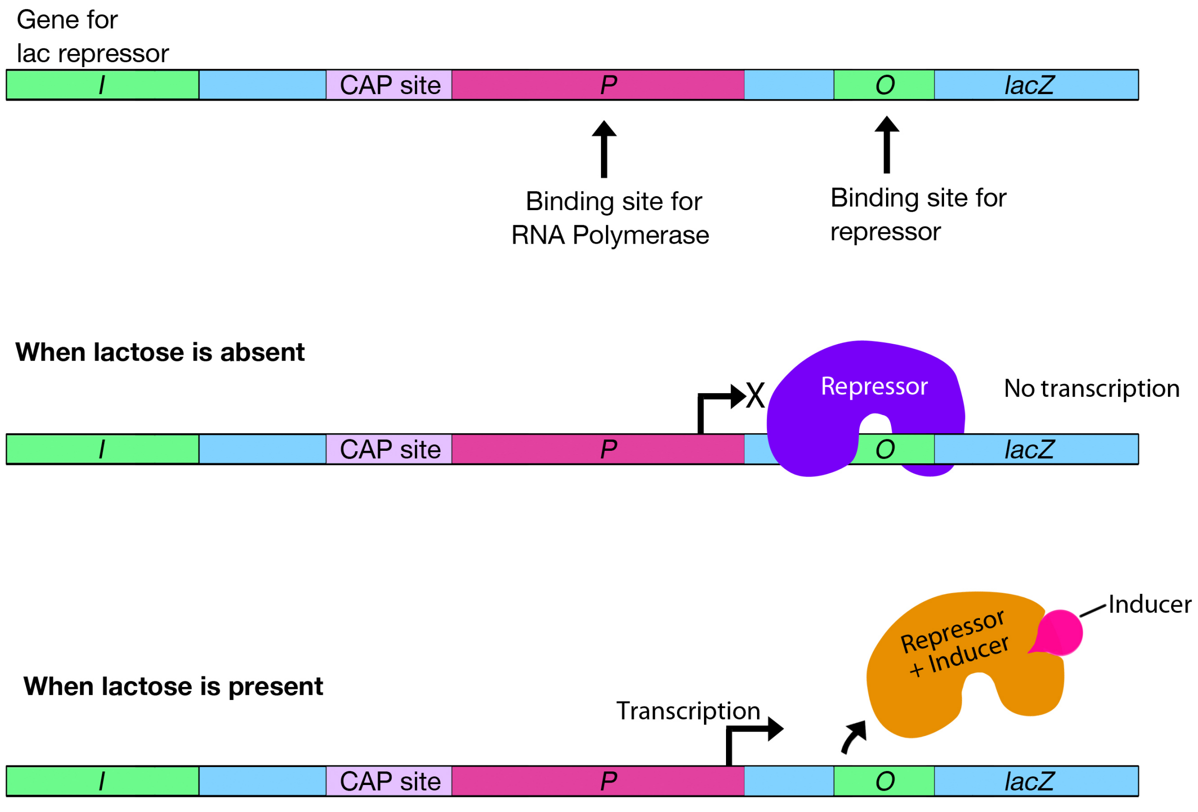

Promoter
Operator
lac I
lac z
lac y
lac a
DNA
Transcription of lac genes results in mRNA
mRNA
Translation of mRNA produces proteins necessary for lactose metabolism
β Galactosidase
Permease
Trans-acetylase

The genes lac z, lac y and lac a are all under the control of a single promoter in the lac operon

We know that promoters indicate where transcription begins, but what determines that a given gene will be transcribed? In addition to the promoter sequences required for transcription initiation, genes have additional regulatory sequences (sequences of DNA on the same DNA molecule as the gene) that control when a gene is transcribed. Regulatory sequences are bound tightly and specifically by transcriptional regulators, proteins that can recognize DNA sequences and bind to them. The binding of such proteins to the DNA can regulate transcription by preventing or increasing transcription from a particular promoter.

Let us first consider an example from prokaryotes. In bacteria, genes are often clustered in groups, such that genes that need to be expressed at the same time are next to each other and all of them are controlled as a single unit by the same promoter. The lac operon, shown below, is one such group of genes that encode proteins needed for the uptake and breakdown of the sugar lactose. The three genes of the lac operon, lac z, lac y and lac a are controlled by a single promoter.

Bacterial cells generally prefer to use glucose for their energy needs, but if glucose is unavailable, and lactose is present, the

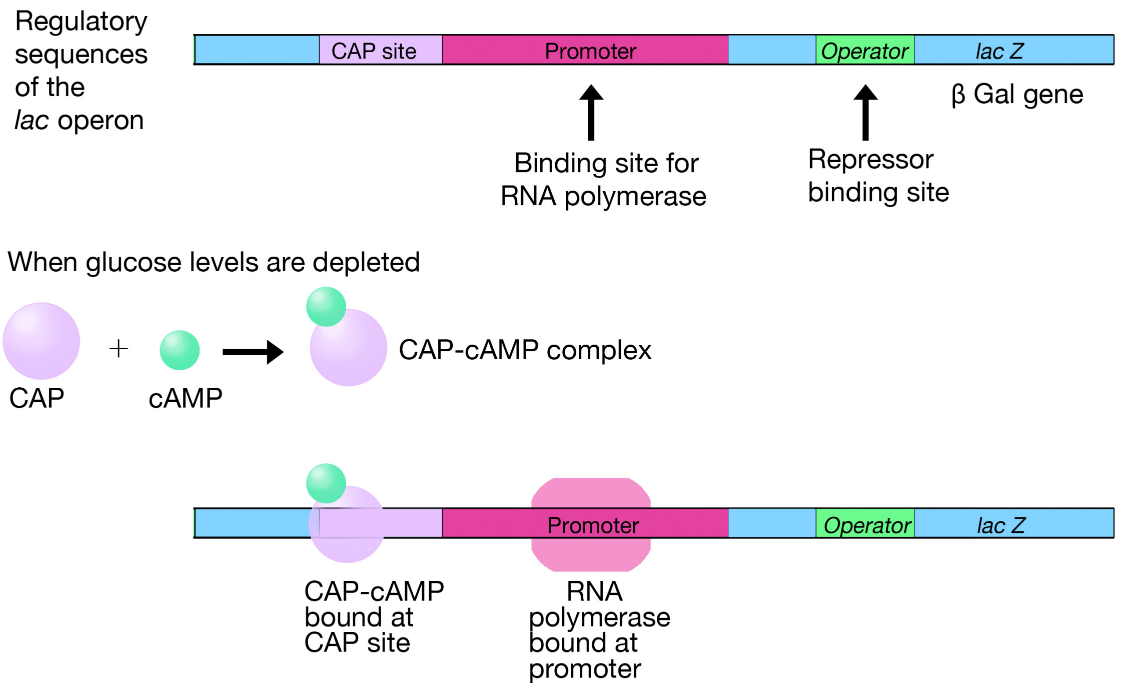

Gene for lac repressor
I
CAP site
P
O
lacZ
Binding site for RNA Polymerase
Binding site for repressor
When lactose is absent
X
Repressor
No transcription
I
CAP site
P
O
lacZ
When lactose is present
Transcription
Repressor + Inducer
Inducer
I
CAP site
P
O
lacZ

Lac Operon Regulation

bacteria will take up lactose and break it down for energy. Since the proteins for taking up and breaking down lactose are only needed when glucose is absent and lactose is available, the bacterial cells need a way to express the genes of the lac operon only under those conditions. At times when lactose is absent, the cells do not need to express these genes.

Watch Kevin’s YouTube lectures on Transcription Regulation HERE, HERE, and HERE

How do bacteria achieve this?
Transcription of the lac cluster of genes is primarily controlled by a repressor protein that binds to a region of the DNA just downstream of the -10 sequence of the lac promoter. Recall that the promoter is where the RNA polymerase must bind to begin transcription. The place where the repressor is bound is called the operator (labeled O in the figure). When the repressor is bound at this position, it physically blocks the RNA polymerase from transcribing the genes, just as a vehicle blocking your driveway would prevent you from pulling out.


Regulatory sequences of the *lac* operon
CAP site
Promoter
Operator
lac Z
β Gal gene
Binding site for RNA polymerase
Repressor binding site
When glucose levels are depleted
CAP
+
cAMP
CAP-cAMP complex
Promoter
Operator
lac Z
CAP-cAMP bound at CAP site
RNA polymerase bound at promoter

Lac Operon Activation

Obviously, if you want to leave, the vehicle that is blocking your path must be removed. Likewise, in order for transcription to occur, the repressor must be removed from the operator to clear the path for RNA polymerase. How is the repressor removed?

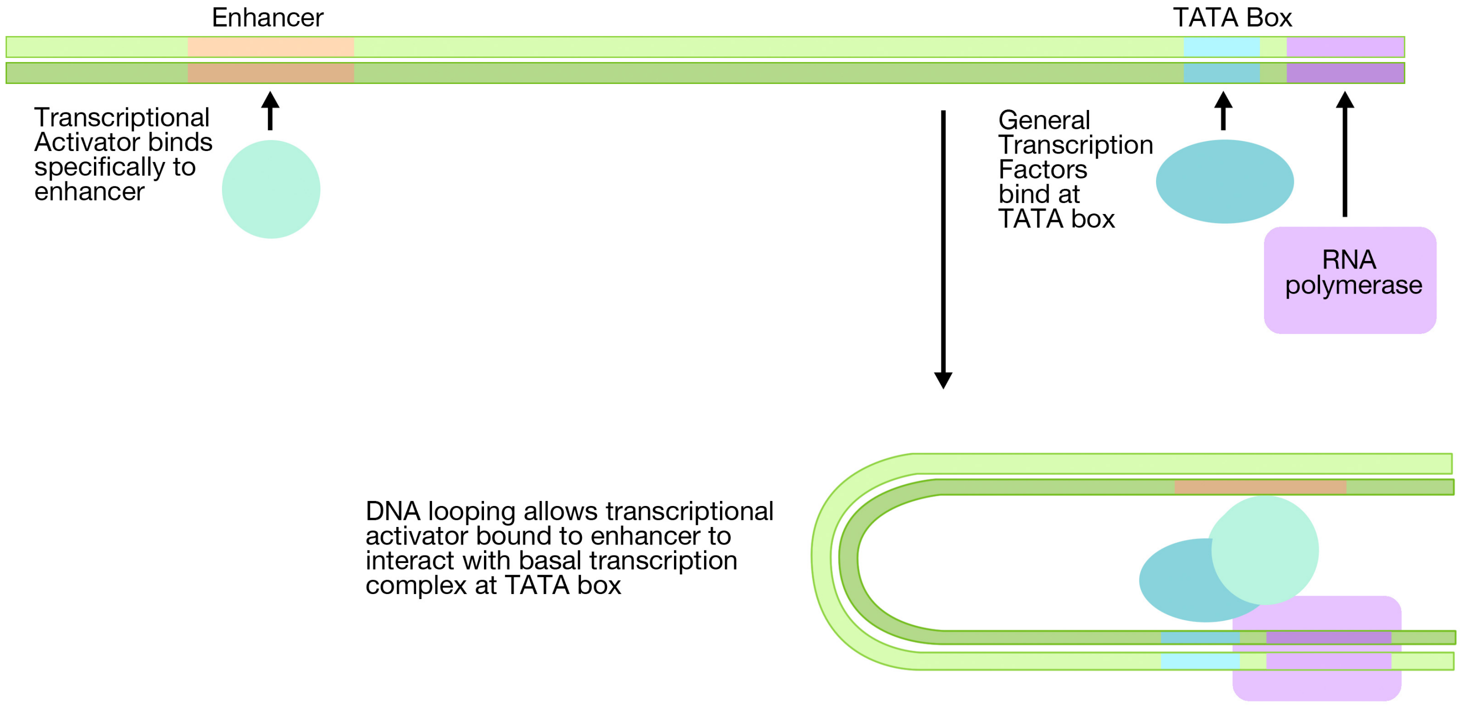
When the sugar lactose is present, it binds to the repressor, changing its conformation so that it no longer binds to the operator. When the repressor is no longer bound at the operator, the "road-block" in front of the RNA polymerase is removed, permitting the transcription of the genes of the lac operon.

Because the binding of the lactose induces the expression of the genes in the lac operon, lactose is called an inducer. (Technically, the inducer is allolactose, a molecule made from lactose by the cell, but the principle is the same.)

What makes this an especially

effective control system is that the genes of the lac operon encode proteins that break down lactose. Turning on these genes requires lactose to be present. Once the lactose is broken down, the repressor binds to the operator once more and the lac genes are no longer expressed. This allows the genes to be expressed only when they are needed.

But how do glucose levels affect the expression of the lac genes? We noted earlier that if glucose was present, lactose would not be used. A second level of control is exerted by a protein called CAP that binds to a site adjacent to the promoter and recruits RNA polymerase to bind the lac promoter. When glucose is depleted, there is an increase in levels of cAMP which binds to CAP. The CAP-cAMP complex then binds the CAP site, as shown in the figure. The combination of CAP binding and the lac repressor dissociating from the operator when lactose levels are high ensures high levels of transcription of the lac operon just when it is most needed. The CAP protein binding may be thought of as a green light for the RNA polymerase, while the removal of repressor is like the lifting of a barricade in front of it. When both conditions are met, the RNA polymerase transcribes the downstream genes.


Enhancer
TATA Box
Transcriptional Activator binds specifically to enhancer
General Transcription Factors bind at TATA box
RNA polymerase
DNA looping allows transcriptional activator bound to enhancer to interact with basal transcription complex at TATA box

Enhancer Mechanism

The lac operon we have just described is a set of genes that are expressed only under the specific conditions of glucose depletion and lactose availability. Other genes may be expressed *unless* a particular condition is met. An example of this is the *trp* operon in bacterial cells, which encodes enzymes necessary for the synthesis of the amino acid tryptophan. These genes are expressed at all times, except when tryptophan is available from the cell's surroundings. This means that these genes must be prevented from being expressed in the presence of tryptophan. This is achieved by having a repressor protein that will bind to the operator only in the presence of tryptophan.

Watch Kevin’s YouTube lectures on Transcription Regulation HERE, HERE, and HERE

Transcription in eukaryotes is also regulated by the binding of proteins to specific DNA sequences, but with some differences from the simple schemes outlined above. For most eukaryotic genes, general transcription factors and RNA polymerase (i.e., the basal transcription complex) are necessary, but not sufficient, for high levels of transcription.

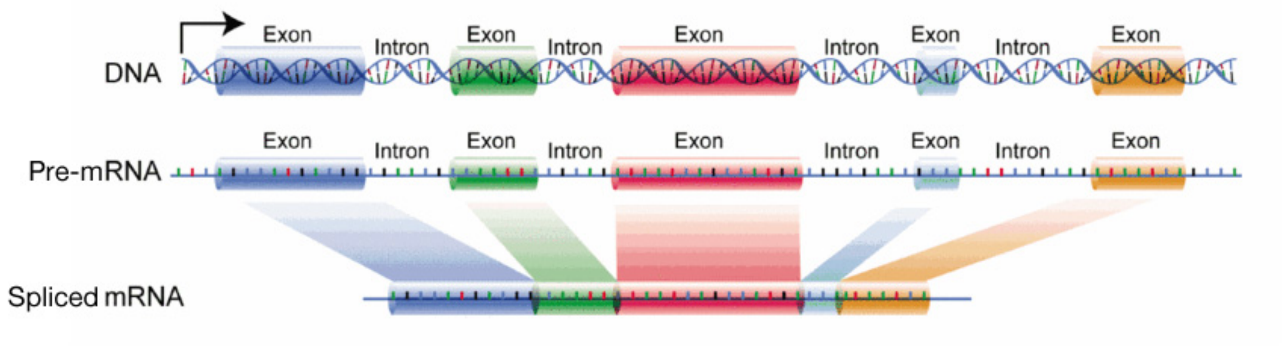
In eukaryotes, additional regulatory sequences called enhancers and the proteins that bind to the enhancers are needed to achieve high levels of transcription. Enhancers are DNA sequences that regulate the transcription of genes. Unlike prokaryotic regulatory sequences, enhancers don't need to be next to the gene they control. Often they are many kilobases away on the DNA. As the name suggests, enhancers can enhance (increase) transcription of a particular gene.

How can a DNA sequence far from the gene being transcribed affect the level of its transcription?

Enhancers work by binding proteins (transcriptional activators) that can, in turn, interact with the proteins bound at the promoter. The enhancer region of the DNA, with its associated transcriptional activator(s) can come in contact with the basal transcription complex that is bound at a distant TATA box by looping of the DNA (previous page). This allows the protein bound at the enhancer to make contact with the proteins in the basal transcription complex.

One way that the transcriptional activator bound to the enhancer increases the transcription from a distant promoter is that it increases the frequency and efficiency with which the basal transcription complex is formed at the promoter.

Another mechanism by which proteins bound at the enhancer can affect transcription is by recruiting to the promoter other proteins that can modify the structure of the chromatin in that region. As we noted earlier, in eukaryotes, DNA is packaged with proteins to form chromatin. When the DNA is tightly associated with these proteins, it is difficult to access for transcription. So proteins that can make the DNA more accessible to the transcription



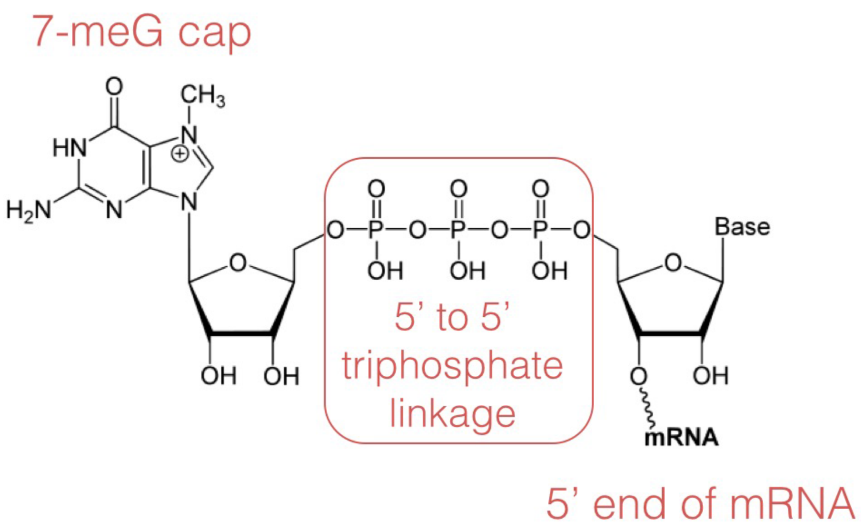
RNA Splicing

machinery can also play a role in the extent to which transcription occurs.

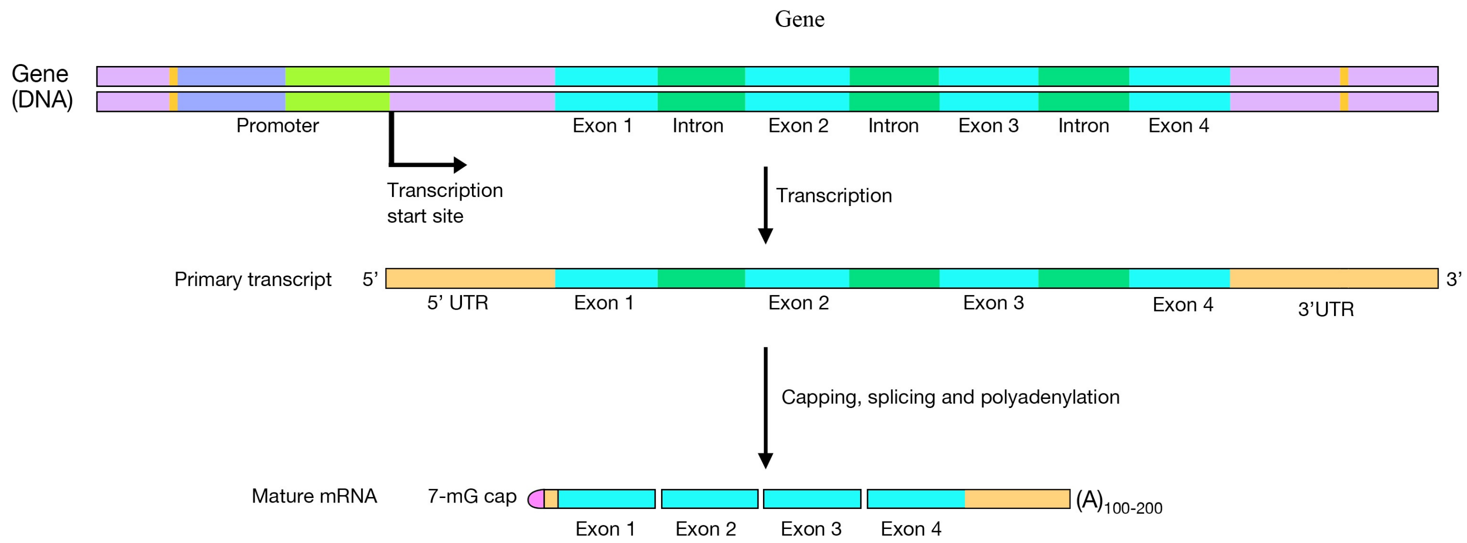
In addition to enhancers, there are also negative regulatory sequences called silencers. Such regulatory sequences bind to transcriptional repressor proteins. Transcriptional activators and repressors are modular proteins- they have a part that binds DNA and a part that activates or represses transcription by interacting with the basal transcription complex.

RNA Processing

So far, we have looked at the mechanism by which the information in genes (DNA) is transcribed into RNA. The newly made RNA, also known as the primary transcript (the product of transcription is known as a transcript) is further processed before it is functional. Both prokaryotes and eukaryotes process their ribosomal and transfer RNAs.

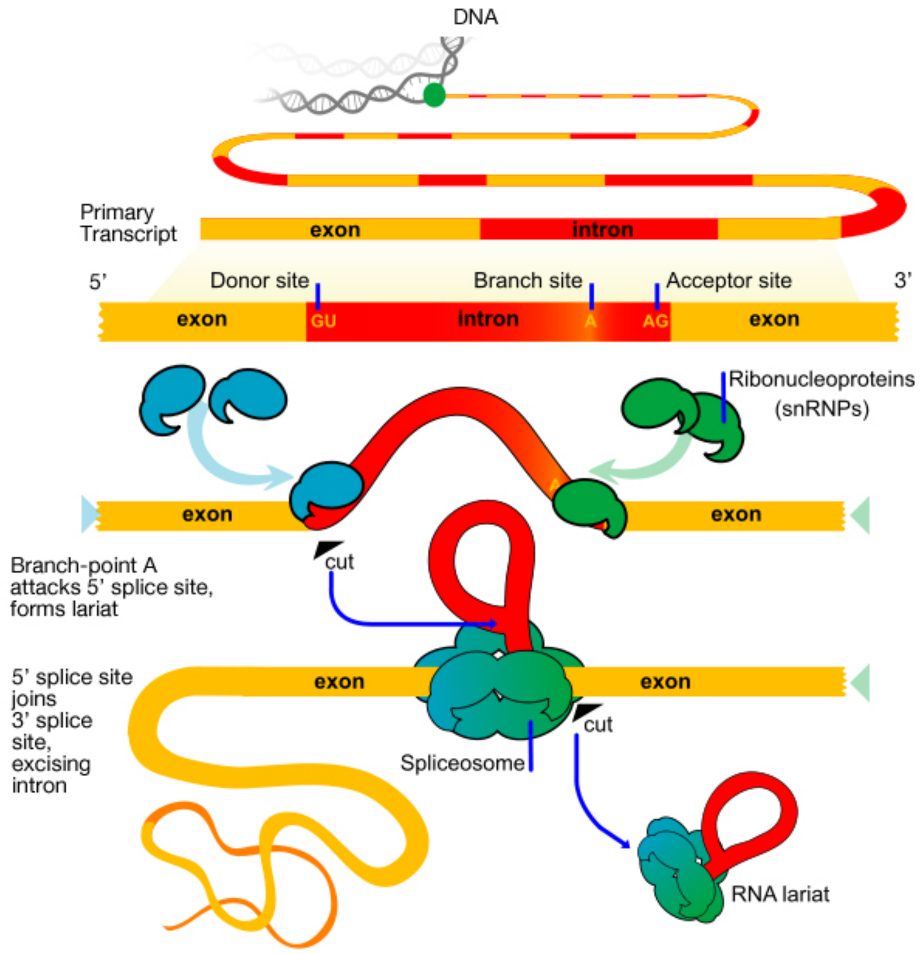

7-meG cap
O
CH3
HN
N
N
H2N
N
N
O
O
O
O
O
P
O
P
O
P
O
OH
OH
OH
Base
O
5' to 5' triphosphate linkage
OH
OH
O
OH
mRNA
5' end of mRNA

mRNA Cap Structure


Gene
Gene (DNA)
Promoter
Exon 1
Intron
Exon 2
Intron
Exon 3
Intron
Exon 4
Transcription start site
Transcription
Primary transcript
5'
3'
5' UTR
Exon 1
Exon 2
Exon 3
Exon 4
3'UTR
Capping, splicing and polyadenylation
Mature mRNA
7-mG cap
(A)100-200
Exon 1
Exon 2
Exon 3
Exon 4

Steps in Processing Eukaryotic Messenger RNAs

The major difference in RNA processing, however, between prokaryotes and eukaryotes, is in the processing of messenger RNAs. We will focus on the processing of mRNAs in this discussion. You will recall that in bacterial cells, the mRNA is translated directly as it comes off the DNA template. In eukaryotic cells, RNA synthesis, which occurs in the nucleus, is separated from the protein synthesis machinery, which is in the cytoplasm. In addition, eukaryotic genes have introns, noncoding regions that interrupt the gene's coding sequence. The mRNA copied from genes containing introns will also therefore have regions that interrupt the information in the gene. These regions must be


DNA
Primary Transcript
exon
intron
5'
Donor site
Branch site
Acceptor site
3'
exon
GU
intron
A
AG
exon
Ribonucleoproteins (snRNPs)
exon
exon
Branch-point A attacks 5' splice site, forms lariat
cut
5' splice site joins 3' splice site, excising intron
exon
exon
cut
Spliceosome
RNA lariat

Splicing

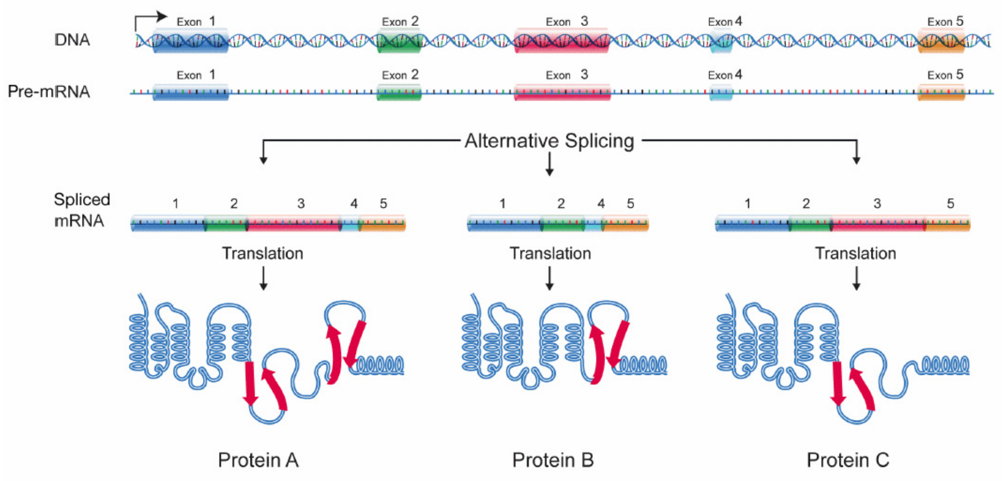
removed before the mRNA is sent out of the nucleus to be used to direct protein synthesis. The process of removing the introns and rejoining the coding sections or exons, of the mRNA, is called splicing. Once the mRNA has been capped, spliced and had a polyA tail added, it is sent from the nucleus into the cytoplasm for translation.

The initial product of transcription of a protein coding gene is called the pre-mRNA (or primary transcript). After it has been processed and is ready to be exported from the nucleus, it is called the mature mRNA or processed mRNA.

What are the processing steps for messenger RNAs?
In eukaryotic cells, pre-mRNAs undergo three main processing steps:

- Capping at the 5' end
- Addition of a polyA tail at the 3' end. and
- Splicing to remove introns

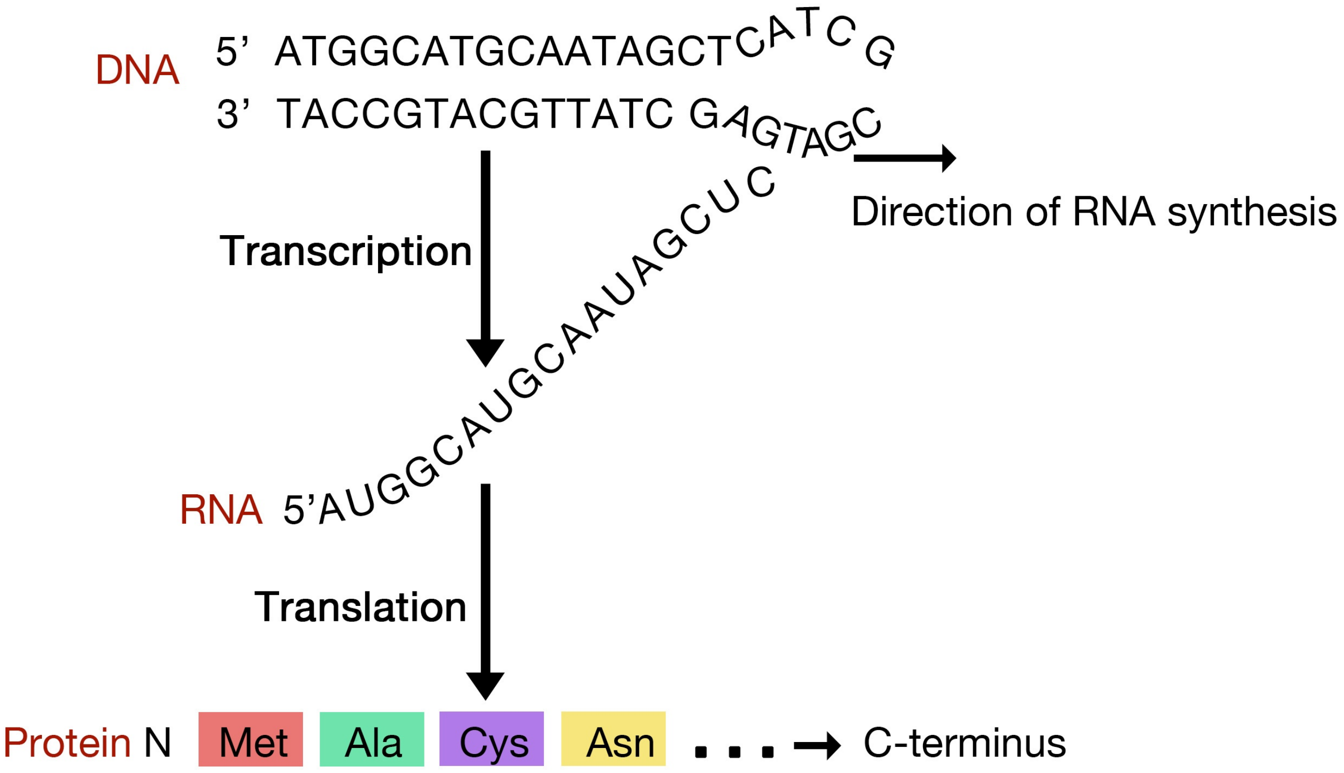
In the capping step of mRNA processing, a 7-methyl guanosine (shown at left) is added at the 5' end of the mRNA. The cap protects the 5' end of the mRNA from degradation by nucleases and also helps to position the mRNA correctly on the ribosomes during protein synthesis.


DNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Pre-mRNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Alternative Splicing
Spliced mRNA
1
2
3
4
5
1
2
4
5
1
2
3
5
Translation
Translation
Translation
Protein A
Protein B
Protein C

Splicing and Protein Diversity

The 3' end of a eukaryotic mRNA is first trimmed, then an enzyme called PolyA Polymerase adds a "tail" of about 200 ‘A’ nucleotides to the 3' end. There is evidence that the polyA tail plays a role in efficient translation of the mRNA, as well as in the stability of the mRNA. The cap and the polyA tail on an mRNA are also indications that the mRNA is complete (i.e., not defective).

Introns are removed from the pre-mRNA by the activity of a complex called the spliceosome. The spliceosome is made up of proteins and small RNAs that are associated to form protein-RNA enzymes called small nuclear ribonucleoproteins or snRNPs (pronounced SNURPS). The splicing machinery must be able to recognize splice junctions (i.e., the end of each exon and the start of the next) in order to correctly cut out the introns and join the exons to make the mature, spliced mRNA.



Coupled Transcription and Translation in Prokaryotes

What signals indicate where an intron starts and ends?

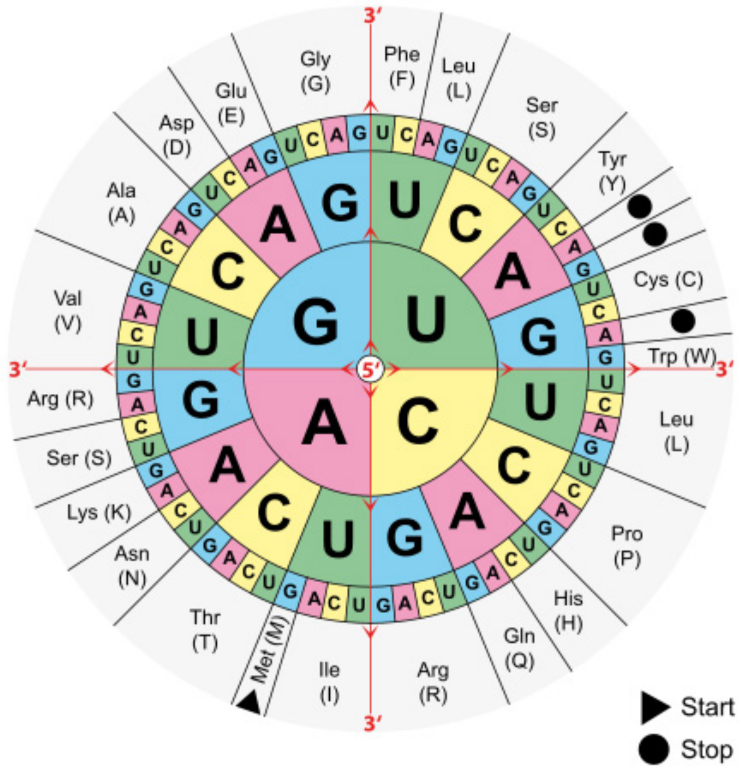
The base sequence at the start (5' or left end, also called the donor site) of an intron is GU while the sequence at the 3' or right end (a.k.a. acceptor site) is AG. There is also a third important sequence within the intron, called a branch point, that is important for splicing.

Watch Kevin’s YouTube lectures on Translation HERE, HERE, and HERE

There are two main steps in splicing:

- In the first step, the pre-mRNA is cut at the 5' splice site (the junction of the 5' exon and the intron). The 5' end of the intron then is joined to the branch point within the intron. This generates the lariat-shaped molecule characteristic of the splicing process

•In the second step, the 3' splice site is cut, and the two exons are



The Genetic Code

joined together, and the intron is released.

Many pre-mRNAs have a large number of exons that can be spliced together in different combinations to generate different mature mRNAs. This is called alternative splicing, and allows the production of many different proteins using relatively few genes, since a single RNA can, by combining different exons during splicing, create many different protein coding messages. Because of alternative splicing, each gene in our DNA gives rise, on average, to three different proteins.

Once protein coding messages have been processed by capping, splicing and addition of a poly A tail, they are transported out of the nucleus to be translated in the cytoplasm.

Translation

Translation is the process by which information in mRNAs is used to direct the synthesis of proteins. As you have learned in introductory biology, in eukaryotic cells, this process is carried out in the

The Codon Song

To the tune of “*When I'm Sixty Four*”

Building of proteins, you oughta know
Needs amino A's
Peptide bond catalysis in ribosomes
Triplet bases, three letter codes
Mixing and matching nucleotides
Who is keeping score?
Here is the low down
If you count codons
You'll get sixty four

Got - to - line - up - right
16-S R-N-A and
Shine Dalgarno site

You can make peptides, every size
With the proper code
Start codons positioned
In the P site place
Initiator t-RNAs
UGA stops and AUGs go
Who could ask for more?
You know the low down

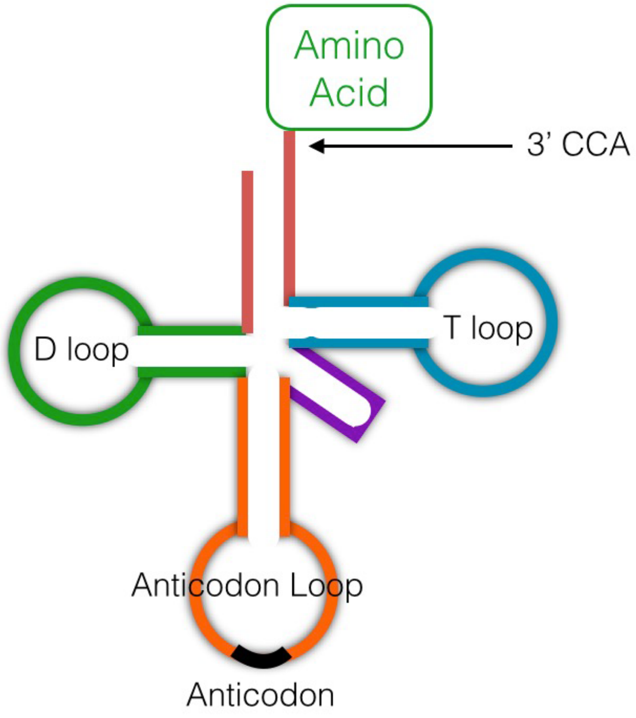
Count up the codons

There are sixty four

Recorded by Tim Karplus
Lyrics by Kevin Ahern

cytoplasm of the cell, by large RNA-protein machines called ribosomes. Ribosomes contain ribosomal RNAs (rRNAs) and proteins. The proteins and rRNAs are organized into two subunits, a large and a small. The large subunit has an enzymatic activity, known as a peptidyl transferase, that makes the peptide bonds that join amino acids to make a polypeptide. The small and large subunits assemble on the mRNA at its 5'end to initiate translation. Ribosomes function by binding to mRNAs and holding them in a way that allows the amino acids encoded by the RNA to be joined sequentially to form a polypeptide.

Watch Kevin's YouTube lectures on Translation HERE, HERE, and HERE

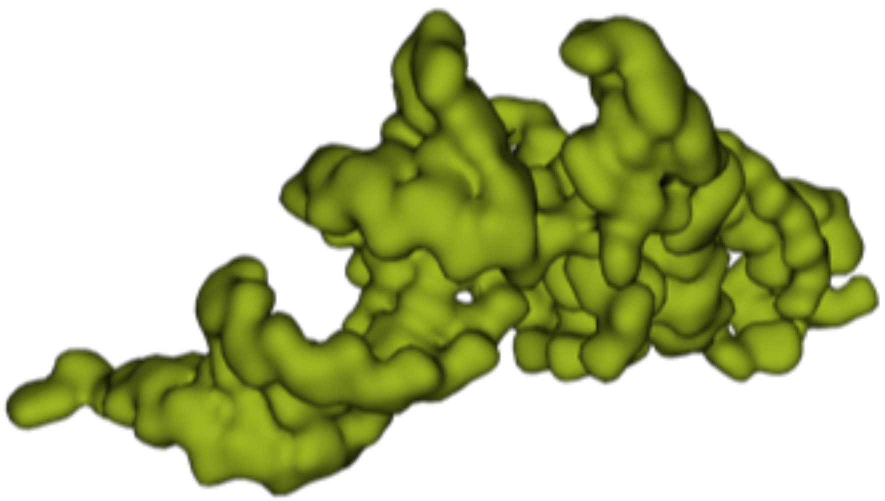


tRNA Structure

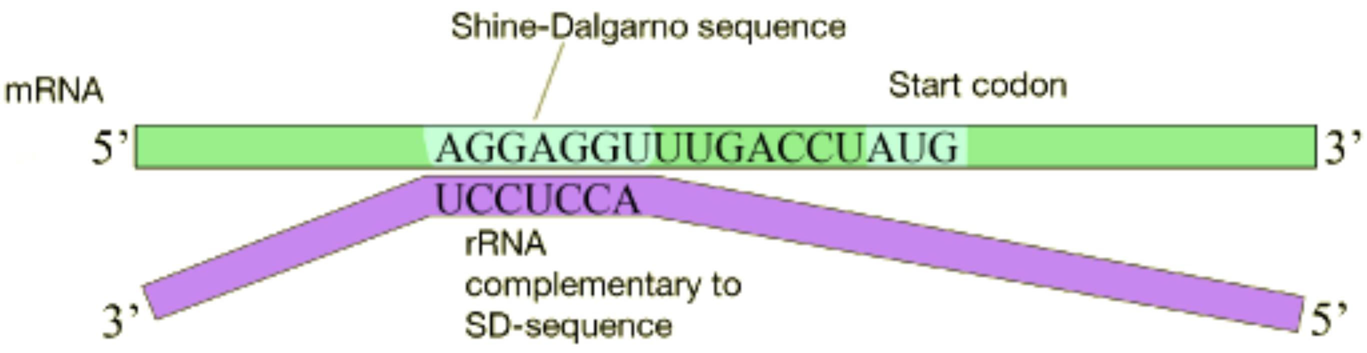
The sequence of an mRNA directly specifies the sequence of amino acids in the protein it encodes. Each amino acid in the protein is specified by a sequence of 3 bases called a codon in the mRNA. For example, the amino acid tryptophan is encoded by the sequence 5'UGG3' on an mRNA.

Given that there are 4 bases in RNA, the number of different 3-base combinations that are possible is 4^3, or 64. There are, however, only 20 amino acids that are used in building proteins. This discrepancy in the number of possible codons and the actual number of amino acids they specify is explained by the fact that the same amino acid may be specified by more than one codon. In fact, with the exception of the amino acids methionine and tryptophan, all the other amino acids are encoded by multiple codons. The

Interactive 5.2



Phenylalanyl-tRNA


Shine-Dalgarno sequence
mRNA
Start codon
5'
AGGAGGUUUGACCUAUG
3'
UCCUCCA
rRNA
complementary to
SD-sequence
3'
5'

mRNA Alignment by Shine-Dalgarno Sequence

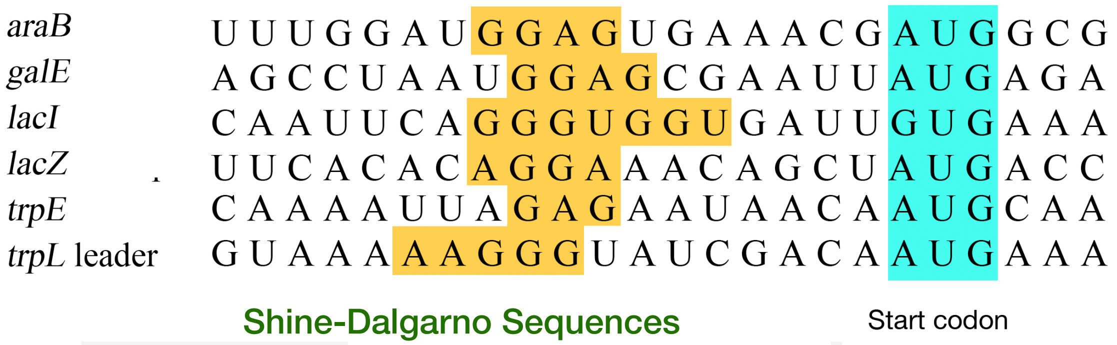
figure above shows the codons that are used for each of the twenty amino acids.

Three of the 64 codons are known as termination or stop codons and as their name suggests, indicate the end of a protein coding sequence. The codon for methionine, AUG, is used as the start, or initiation, codon.

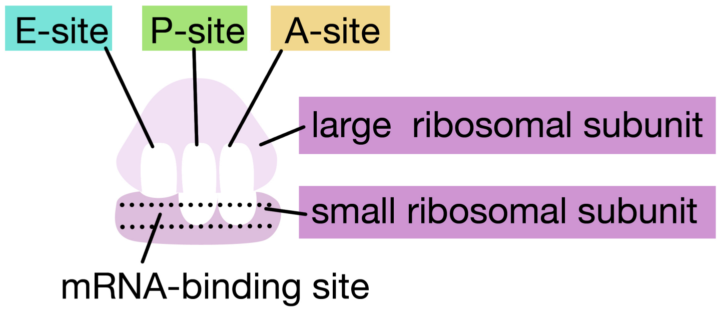
This ingenious system is used to direct the assembly of a protein in the same way that you might string together colored beads in a particular order using instructions that used symbols like 111 for a red bead, followed by 222 for a green bead, 333 for yellow, and so on, till you came to 000, indicating that you should stop stringing beads.

While the ribosomes are literally the protein factories that join amino acids together using the instructions in mRNAs, another class of RNA molecules, the transfer RNAs (tRNAs) are also needed for translation. Transfer RNAs (see figure, left) are small RNA molecules, about 75-80 nucleotides long, that function to 'interpret' the instructions in the mRNA during protein synthesis. In terms of the bead analogy above, someone, or something, has to be able to bring a red bead in when the instructions indicate 111, and a green bead when the instructions say 222. Unlike a human, who can choose a red bead when 111 is present in the instructions, neither ribosomes nor tRNAs can think. The system, therefore, relies, like so many processes in cells, solely on molecular recognition.

A given transfer RNA is specific for a particular amino acid. It is linked covalently to this amino acid at its 3' end by an enzyme called aminoacyl tRNA synthetase. There is an aminoacyl tRNA synthetase specific for each amino acid. A tRNA with an amino acid attached to it is said to be charged. Another region of the tRNA has a sequence of 3 bases, the anticodon, that is


araB UUUGGAUGGAGUGAAACGAUGGCG
galE AGCCUAAUGGAGCGAAUUAUGAGA
lacI CAAUUCAGGGUGGUGAUUGUGAAA
lacZ UUCACACAGGAAACAGCUAUGACC
trpE CAAAAUUAGAGAAUAACAAUGCAA
trpL leader GUAAAAAGGGUAUCGACAAUGAAA
Start codon

Shine-Dalgarno Sequences

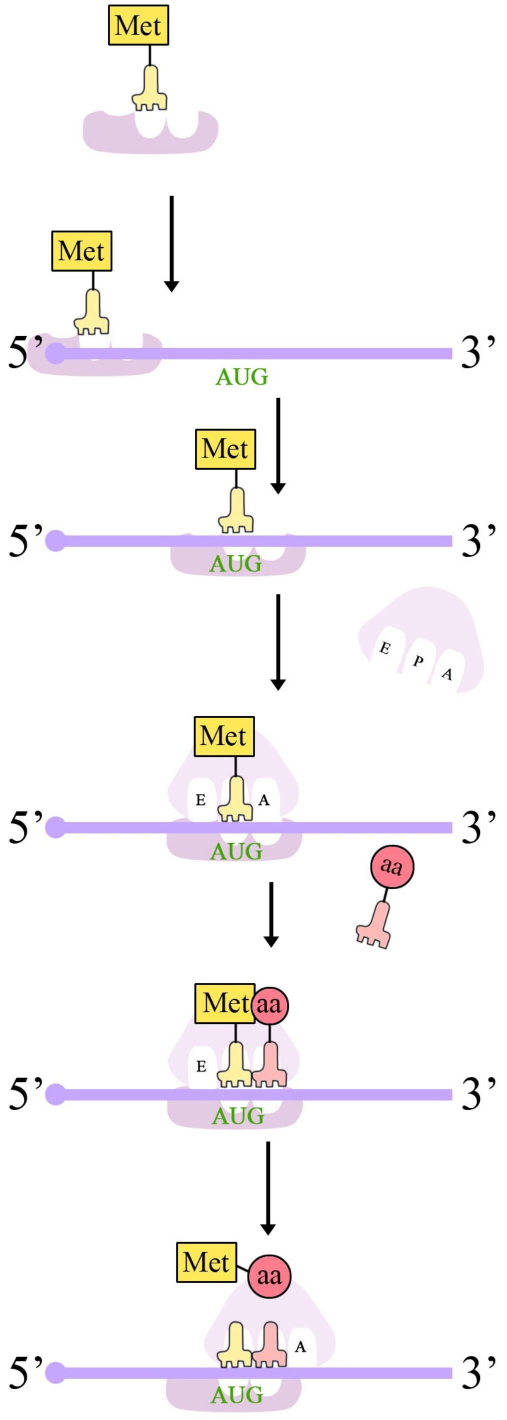


A,P and E Sites in the Ribosome

complementary to the codon for the amino acid it is carrying. When the tRNA encounters the codon for its amino acid on the messenger RNA, the anticodon will base-pair with the codon, and the amino acid attached to it will be brought in to the ribosome to be added on to the growing protein chain.

With an idea of the various components necessary for translation we can now take a look at the process of protein synthesis. The main steps in the process are similar in prokaryotes and eukaryotes. As we already noted, ribosomes bind to mRNAs and facilitate the interaction between the codons in the mRNA and the anticodons on charged tRNAs.

In bacterial cells, translation is coupled with transcription and begins even before the mRNA has been completely synthesized. How does the ribosome recognize and bind to the mRNA? Many



Charged initiator tRNA and small ribosomal subunit

Small ribosomal subunit binds to ribosome binding site on mRNA

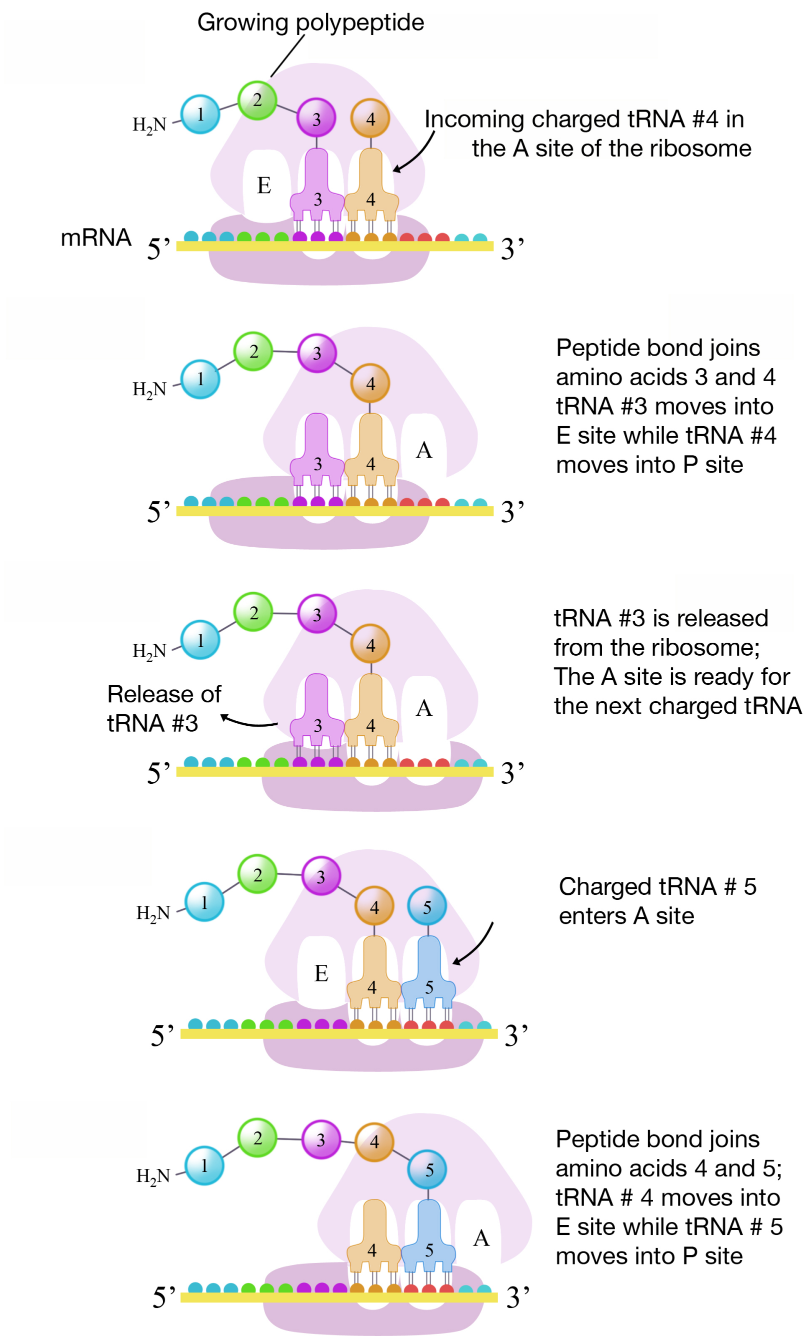
Methionine-charged tRNA binds to the start codon (AUG)

Large ribsomal subunit joins initiation complex. Initiator tRNA occupies the P-site

Second tRNA corresponding to the next codon occupies A-site

A peptide bond is made between the methionine and the amino acid on the second tRNA; initiator tRNA moves to E-site, second tRNA moves to P-site

Initiation of Translation


Growing polypeptide
H_2N
1
2
3
4
E
Incoming charged tRNA #4 in the A site of the ribosome
mRNA
5’
3’
Peptide bond joins amino acids 3 and 4 tRNA #3 moves into E site while tRNA #4 moves into P site
A
tRNA #3 is released from the ribosome; The A site is ready for the next charged tRNA
Release of tRNA #3
Charged tRNA # 5 enters A site
5
Peptide bond joins amino acids 4 and 5; tRNA # 4 moves into E site while tRNA # 5 moves into P site

Elongation of Translation

The Ribosome

To the tune of “*America the Beautiful*”

O beautiful with R-N-A
That makes the peptide bonds
You hold t-RNA so it
Can pair up with co-dons

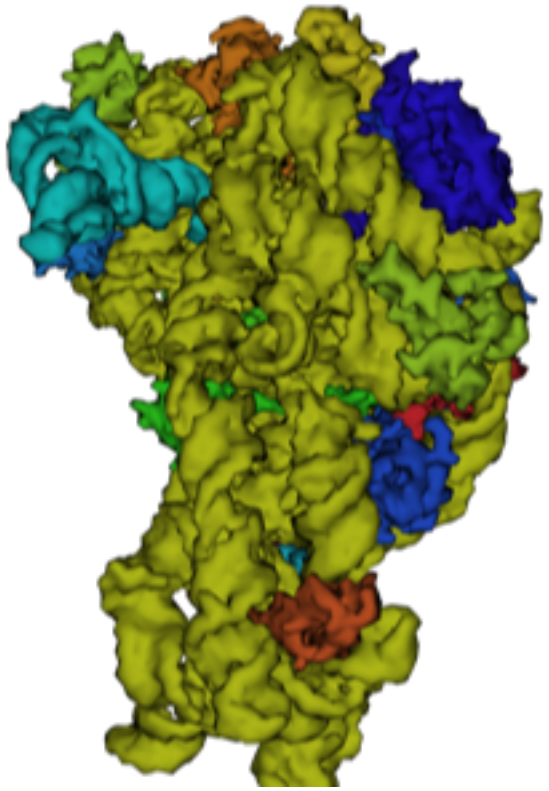
The Ribosome! The Ribosome!
Translate m-RNA
Initiate and translocate
From start to U-G-A

Recorded by Tim Karplus
Lyrics by Kevin Ahern

bacterial mRNAs carry a short purine-rich sequence known as the Shine-Dalgarno site upstream of the AUG start codon, as shown in the figure below. This sequence is recognized and bound by a complementary sequence in the 16S rRNA that is part of the small ribosomal subunit as shown above. Because the Shine-Dalgarno site serves to recruit and bind the ribosome, it is also referred to as the Ribosome Binding Site or RBS.

A variation of this process of ribosome assembly operates in eukaryotic cells. We already know that in eukaryotic cells, processed mRNAs are sent from the nucleus to the cytoplasm.

Interactive 5.3



The Ribosome

The small and large subunits of ribosomes, each composed of characteristic rRNAs and proteins are found in the cytoplasm and assemble on mRNAs to form complete ribosomes that carry out translation.

Protein synthesis in eukaryotes starts with the binding of the small subunit of the ribosome to the 5' end of the mRNA. The assembly of the translation machinery begins with the binding of the small ribosomal subunit to the 7-methyl guanosine cap on the 5'end of an mRNA. Meanwhile, the initiator tRNA pairs with the start codon. (Recall that the start codon is AUG, and codes for methionine. The initiator tRNA carries the amino acid methionine). The large subunit of the ribosome then joins the complex, which is now ready to start protein synthesis.

Ribosomes have two sites for binding charged tRNAs, each of which is positioned to make two adjacent codons on the mRNA available for binding by tRNAs. The initiation codon occupies the first of these ribosomal sites, the P-site. The anticodon complementary to this is on the initiator tRNA, which brings in the first amino acid of the protein. This initial phase of translation is called initiation and requires the help of protein factors called initiation factors.

The second codon of the mRNA is positioned adjacent to the second site on the ribosome, the A site. This is where the tRNA carrying the amino acid specified by the second codon binds. The binding of aminoacyl tRNA to the A-site is mediated by proteins called elongation factors and requires the input of energy. Once the appropriate charged tRNAS have "docked" on the codons by base-pairing between the anticodon on the tRNA and the codon on the mRNA, the ribosome joins the amino acids carried by the two tRNAs by making a peptide bond (see figure at right).

Interestingly, the formation of the peptide bond is catalyzed by a catalytic RNA (the 23S rRNA in prokaryotes) rather than by a protein enzyme.

This
and

Central Dogma Zen- Part 2

To the tune of “*Those Were The Days*”

An organism's cellular construction
With blueprints for the things they have to do
Requires converting DNA instructions
To ribopolymers, oh yes it's true

Because they've been bestowed
With a genetic code
The RNAs provide the cell with means
To link amino A's
In most directed ways
Inside the protein-making cell machines

If "coli" cells don't have galactosidase
And lactose should appear inside its food
The lac repressor leaves the operator
'Cause otherwise metabolism's screwed

Polymerase unwinds
The DNAs it binds
Adjacent to the start site where it docks
Unravels A's and T's
With such amazing ease
At the promoter's little TATA box

The process moves along without much trouble
While making RNA inside the cell
It all occurs inside transcription bubbles
Where bases get linked anti-parallel

mRNA then roams
To find some ribosomes
Subunits large and small bind near the end
The A-U-G's in place
Inside the P site space
Initiation you can comprehend

The mechanism shifts to elongation
Proceeding by three bases at a stretch
A GTP's required for translocation
Advancing 5 to 3 the whole complex

The process moves anon
Until a stop codon
Arrives and causes movement to suspend
Translation has to cease
A peptide gets released
And we have reached the central dogma's end

Recorded by Tim Karplus
Lyrics by Kevin Ahern

subsequent steps in the synthesis of the polypeptide are called the elongation phase of translation. Once the first two amino acids are linked , the first tRNA dissociates, and moves out of the P-site and into the E, or Exit site. The second tRNA then moves into the P-site, vacating the A-site for the tRNA corresponding to the next codon.

The process repeats till the stop codon is in the A-site. At this point, a release factor binds at the A-site, adds a water molecule to the polypeptide at the P-site, and releases the completed polypeptide from the ribosome, which itself, then dissociates into subunits.

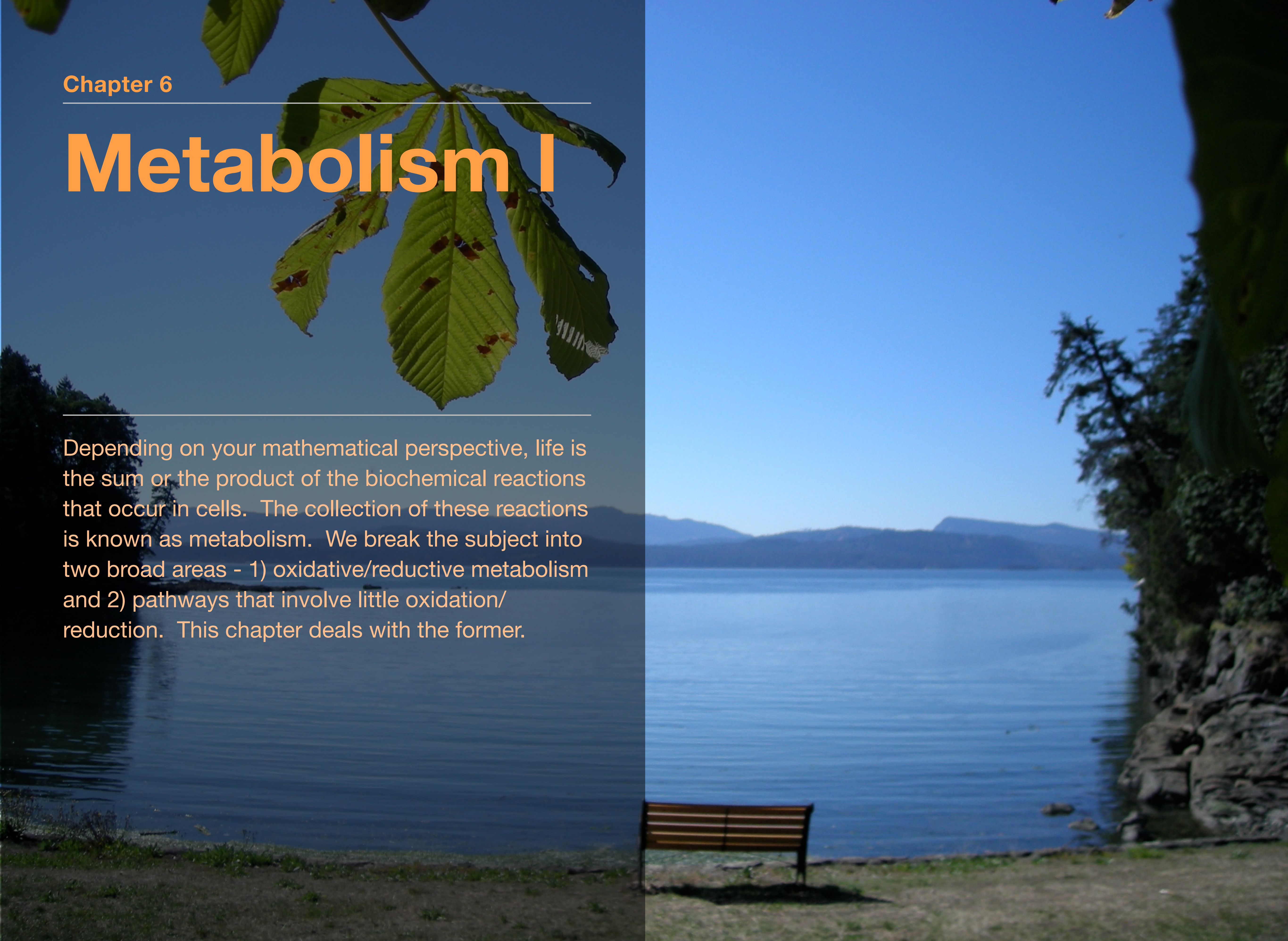
As described in Chapter 3, polypeptides made in this way are then folded into their three dimensional shapes, post-translationally modified and delivered to the appropriate cellular compartments to carry out their functions.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Chapter 6

Metabolism I



Depending on your mathematical perspective, life is the sum or the product of the biochemical reactions that occur in cells. The collection of these reactions is known as metabolism. We break the subject into two broad areas - 1) oxidative/reductive metabolism and 2) pathways that involve little oxidation/ reduction. This chapter deals with the former.

Section 1

Metabolism I

Definitions

Perspectives

Glycolysis

- Intermediates
- Reactions
- Enzymes/Control
- Pyruvate Metabolism

Gluconeogenesis
Citric Acid Cycle
Glyoxylate Pathway
Acetyl-CoA Metabolism
Cholesterol Metabolism
Ketone Body Metabolism
Prostaglandin Synthesis
Fatty Acid Oxidation

- Oxidation of Odd-Chain Fatty Acids
- Unsaturated Fatty Acid Oxidation
- Enzymes of Beta Oxidation
- Alpha Oxidation

Fatty Acid Synthesis

- Enzymes of Fatty Acid Synthesis
- Elongation of Fatty Acids
- Desaturation of Fatty Acids

Metabolism of Fat
Connections to Other Pathways

The cost of living is energy and the producers and consumers of energy in the cell are the chemical reactions known collectively as metabolism. Metabolic processes are governed by the same laws of energy as the rest of the universe, so they must be viewed in the light of Gibbs free energy.

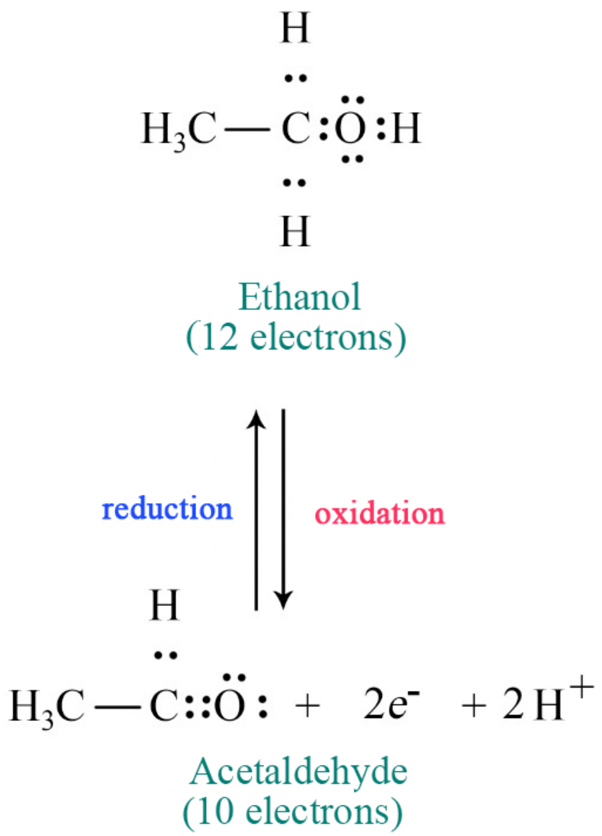
For the most part, the drivers of changes in Gibbs free energy are changes in concentration of reactants and products, but for some reactions, the concentration changes required to run a reaction in the desired direction are not practical. In such cases, cells may use alternative strategies, such as energy coupling reactions (combining an energetically unfavorable reaction with a favorable one, such as the hydrolysis of ATP) to help "drive" the unfavorable reaction. In other cases, cells use alternate pathways around energetically unfavorable reactions.

Definitions

We start by defining a few terms.

Anabolic processes refer to collections of biochemical reactions that make bigger molecules from smaller ones. Examples include the synthesis of **fatty acids** from acetyl-CoA, of proteins from amino acids, of **complex carbohydrates** from simple

A student really ought to know
The ways in which electrons flow
When running catabolically
Removal yields some energy
If anabolic bliss ensues
The tiny charges then reduce


H
H₃C — C:O:H
H
Ethanol
(12 electrons)
reduction
oxidation
H
H₃C — C::O: + 2e⁻ + 2 H⁺
Acetaldehyde
(10 electrons)

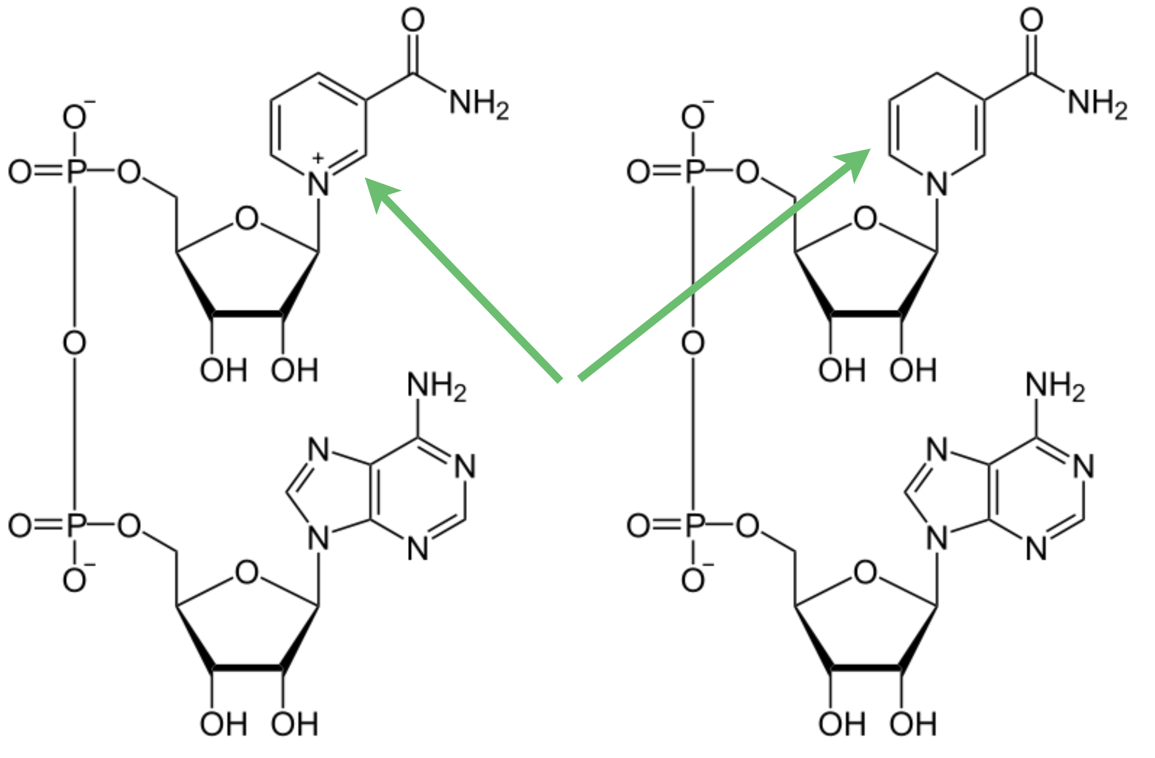
Redox Reactions

sugars, and of nucleic acids from nucleotides. Just as any construction project requires energy, so, too, do anabolic processes require input of energy. Anabolic processes tend to be reductive in nature, in contrast to **catabolic processes**, which are oxidative. Not all anabolic processes are reductive, though. Protein synthesis and nucleic acid synthesis do not involve reduction, though the synthesis of amino acids and nucleotides does.

Catabolic processes are the primary sources of energy for heterotrophic organisms and they ultimately power the anabolic processes. Examples include glycolysis (breakdown of glucose), the citric acid cycle, and fatty acid oxidation. Reductive processes require electron sources, such as **NADPH**, **NADH**, or **$FADH_2$**. Oxidative processes require electron carriers, such as **NAD^+**, **$NADP^+$**, or **FAD**. Catabolic processes are ultimately the source of **ATP** energy in cells, but the vast majority of ATP in heterotrophic organisms is not made in directly in these reactions. Instead, the electrons released by oxidation are collected by electron carriers which donate them, in the mitochondria, to complexes that make ATP (ultimately) by **oxidative phosphorylation**.

Click HERE, HERE, and HERE for Kevin's YouTube lectures on Metabolism and Glycolysis

In our tour of metabolism, we will tackle in this chapter processes that are the most oxidative/reductive in nature and in the following chapter those pathways that involve less reduction/


O
NH₂
O⁻
O=P—O
N⁺
O
OH OH
O
NH₂
N
N
N
N
O=P—O
O⁻
O
OH OH
O
NH₂
O⁻
O=P—O
N
O
OH OH
O
NH₂
N
N
N
N
O=P—O
O⁻
O
OH OH

NAD^+ NADH

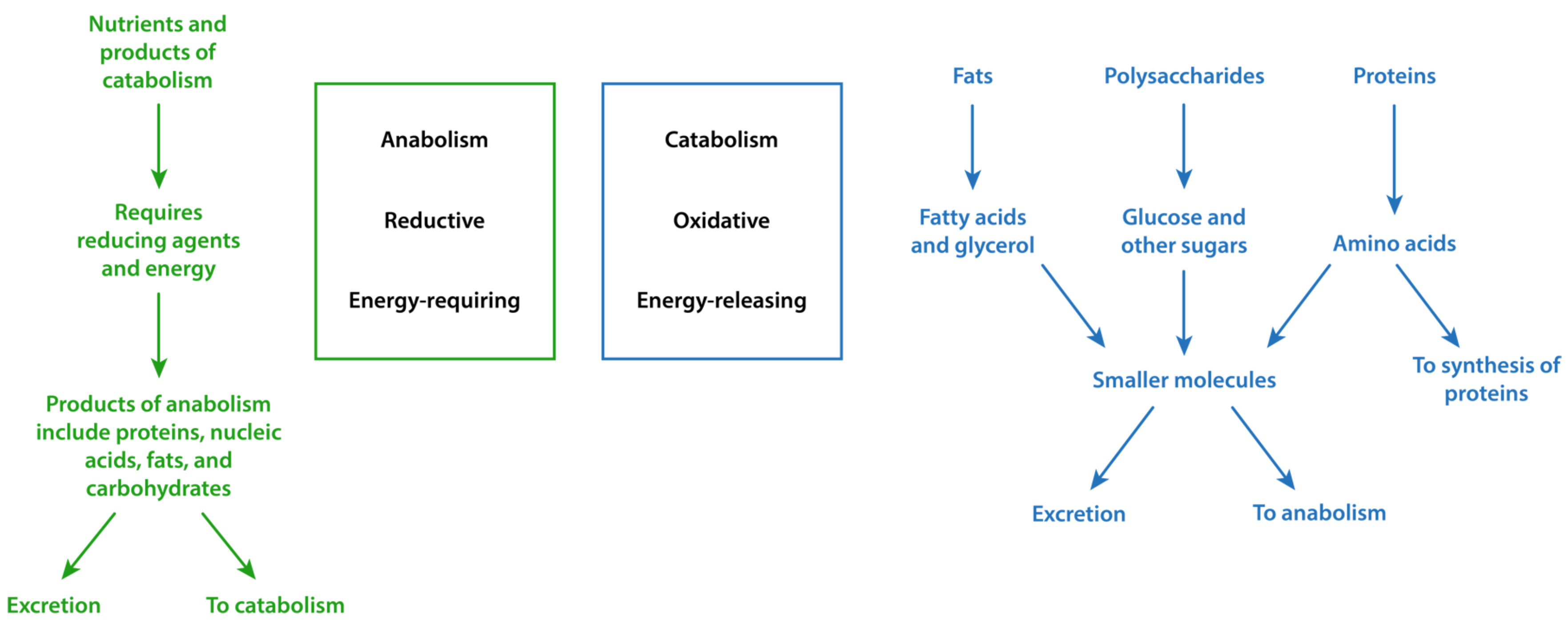
oxidation. The aim in this coverage is not to go through the step-by-step reactions of the pathway, but rather to focus on control points, interesting enzymes, molecules common between pathways, and how the metabolic pathways meet the organism’s needs.

Perspective

We can view metabolism at several levels. At the highest level, we have nutrients, such as sugars, fatty acids and amino acids entering cells and carbon dioxide and other waste products (such as urea) exiting. Cells use the incoming materials for energy and substance to synthesize sugars, nucleotides, and other amino acids as building blocks for the carbohydrates, nucleic acids, fatty compounds, and proteins necessary for life. As we zoom in, we can imagine pathways made up of reactions for breakdown and synthesis of each of these compounds. The figure at left shows such a simple schematic and how the pathways are not isolated from each other – molecular products of one are substrates for another. At a deeper level, we can study individual reactions and discover the enormous complexity and commonality of metabolic reactions.

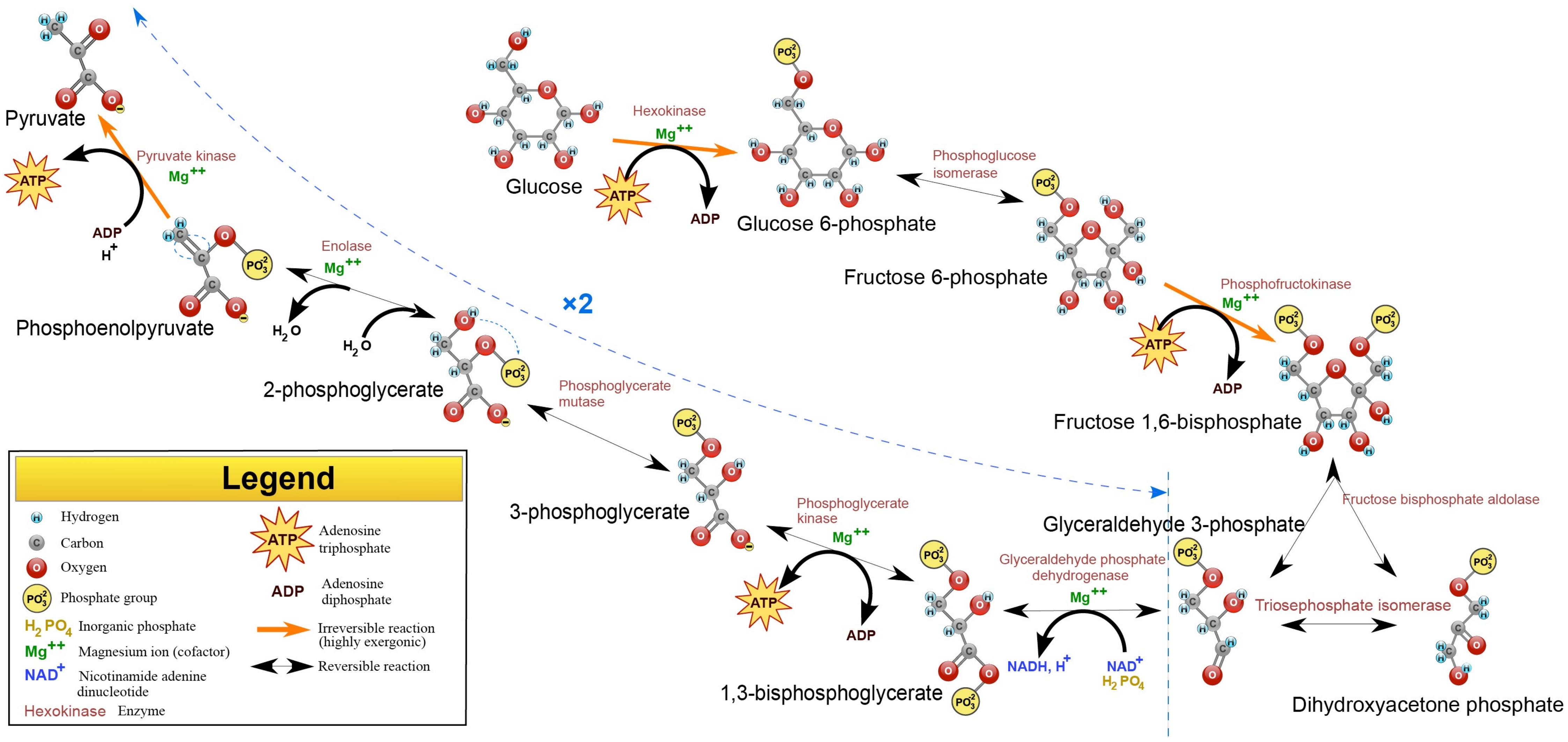
In studying metabolism, we recognize that metabolic pathways are manmade concepts with artificial boundaries. Students commonly think of the molecules in the pathways being tied exclusively to those individual pathways, but with the exception of reactions that have physical barriers (such as those occurring within an organelle), **metabolic pathways** have many common intermediates used in multiple reactions occurring in the same location at the same time and thus cannot be ascribed to any one pathway. The best we can do is understand general directions of pathways in cells.

Anabolic Versus Catabolic Processes


Nutrients and products of catabolism
Requires reducing agents and energy
Products of anabolism include proteins, nucleic acids, fats, and carbohydrates
Excretion
To catabolism
Anabolism
Reductive
Energy-requiring
Catabolism
Oxidative
Energy-releasing
Fats
Polysaccharides
Proteins
Fatty acids and glycerol
Glucose and other sugars
Amino acids
Smaller molecules
To synthesis of proteins
Excretion
To anabolism

Glycolysis

The metabolic pathway traditionally covered first in biochemistry texts is


Pyruvate
ATP
Pyruvate kinase
Mg++
ADP
H+
Phosphoenolpyruvate
Enolase
Mg++
H2O
H2O
2-phosphoglycerate
Glucose
Hexokinase
Mg++
ATP
ADP
Glucose 6-phosphate
Phosphoglucose isomerase
Fructose 6-phosphate
×2
Phosphofructokinase
Mg++
ATP
ADP
Fructose 1,6-bisphosphate
Phosphoglycerate mutase
3-phosphoglycerate
Phosphoglycerate kinase
Mg++
ATP
ADP
1,3-bisphosphoglycerate
Glyceraldehyde phosphate dehydrogenase
Mg++
NADH, H+
NAD+
H2 PO4
Glyceraldehyde 3-phosphate
Fructose bisphosphate aldolase
Triosephosphate isomerase
Dihydroxyacetone phosphate
Legend
Hydrogen
Carbon
Oxygen
Phosphate group
H2 PO4 Inorganic phosphate
Mg++ Magnesium ion (cofactor)
NAD+ Nicotinamide adenine dinucleotide
Hexokinase Enzyme
ATP Adenosine triphosphate
ADP Adenosine diphosphate
Irreversible reaction (highly exergonic)
Reversible reaction

The Reactions of Glycolysis

from Wikipedia

glycolysis and there seems to be no reason to break that trend here. Glycolysis, which literally means "breakdown of sugar," is a catabolic process in which six carbon sugars (**hexoses**) are oxidized and broken down into pyruvate molecules. The corresponding anabolic pathway by which glucose is synthesized is termed **gluconeogenesis**. Both glycolysis and gluconeogenesis are not major oxidative/reductive processes by themselves, with one step in each one involving loss/gain of electrons, but the product of glycolysis, pyruvate, can be completely oxidized to carbon dioxide. Indeed, without

Your cells may have a mounting crisis
Should they not go through glyco-lye-sis
No glucose energy releases
Unless it’s fractured into pieces

production of pyruvate from glucose in glycolysis, a major energy source for the cell is not available. By contrast, gluconeogenesis can synthesize glucose reductively from very simple materials, such as pyruvate and acetyl-CoA/**glyoxylate** (at least in plants). For these reasons we include these pathways in the red/ox collection.

Click HERE, HERE, and HERE for Kevin’s YouTube lectures on Metabolism and Glycolysis

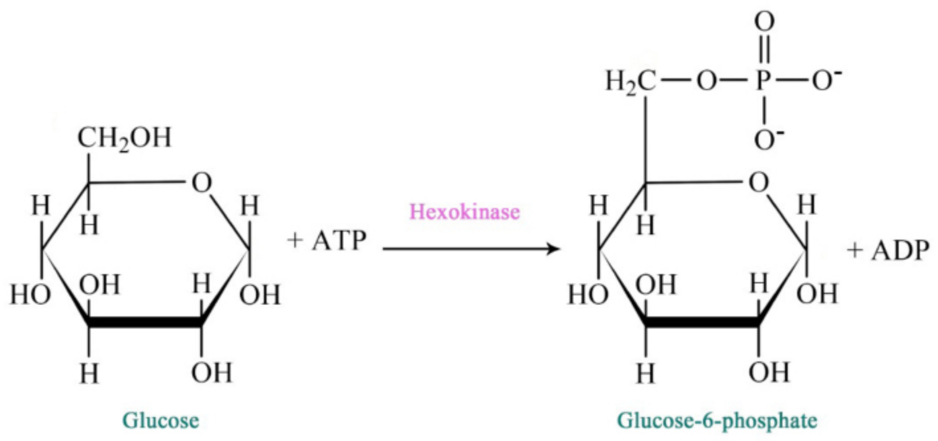
Glucose is the most abundant hexose in nature and is the one people typically associate with glycolysis, but fructose (in the form of fructose-6-phosphate) is metabolized in the cell and galactose can easily be converted into glucose for catabolism in the pathway as well. The end metabolic products of the pathway are two molecules of ATP, two molecules of NADH and two molecules of pyruvate, which, in turn, can be oxidized further in citric acid cycle.

Intermediates

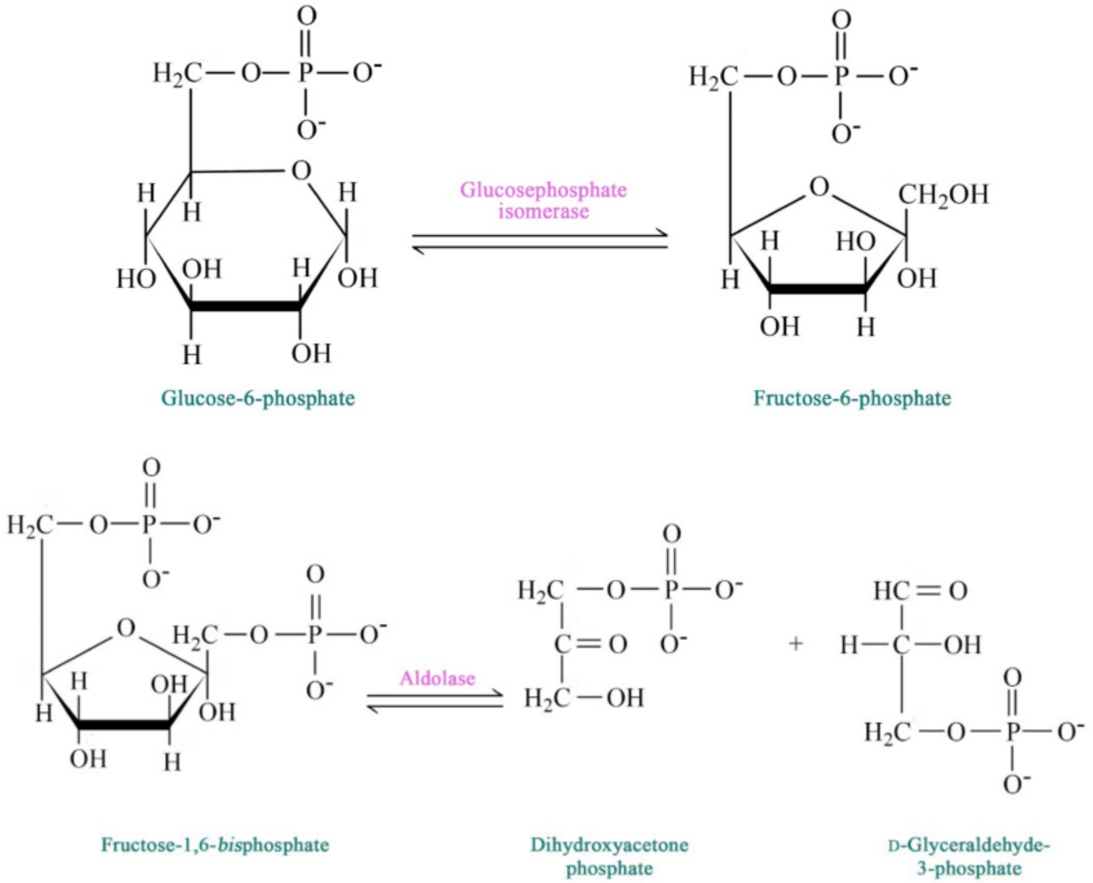
Glucose and **fructose** are the sugar ‘funnels’ serving as entry points to the glycolytic pathway. Other sugars must be converted to either of these forms to be directly metabolized. Some pathways, including the **Calvin Cycle** and the **Pentose Phosphate Pathway** (PPP, see below) contain intermediates in common with glycolysis, so in that sense, almost any cellular sugar can be metabolized here. Intermediates of glycolysis that are common to other pathways include **glucose-6-phosphate** (PPP, glycogen metabolism), F6P (PPP), **G3P** (Calvin, PPP), **DHAP** (PPP, glycerol metabolism, Calvin), 3PG (Calvin, PPP), **PEP** (C4 plant metabolism, Calvin), and **pyruvate** (fermentation, acetyl-CoA genesis, amino acid metabolism).

Reactions

The pathway of glycolysis begins with two inputs of energy. First, glucose gets a phosphate from ATP to make glucose-6-phosphate (**G6P**) and later **fructose-6-phosphate** (**F6P**) gets another phosphate from ATP to make **fructose-1,6-bisphosphate** (**F1,6BP**).



Step 1 of Glycolysis

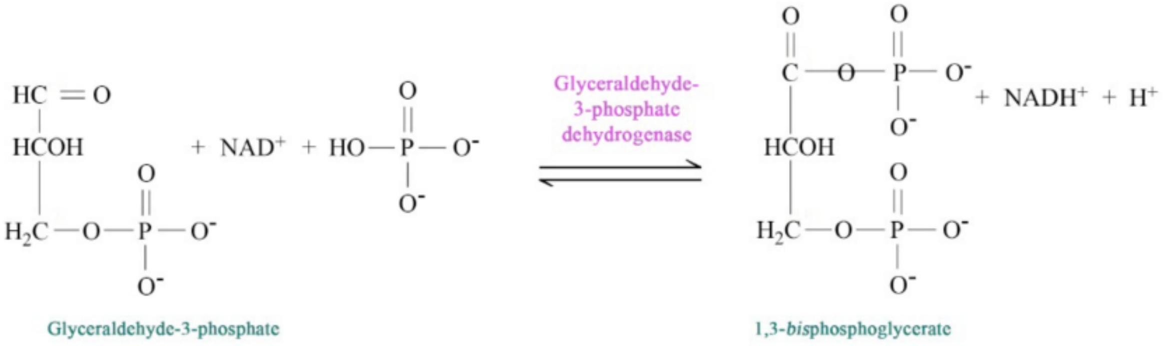

Glucosephosphate isomerase
Glucose-6-phosphate
Fructose-6-phosphate
Aldolase
Fructose-1,6-bisphosphate
Dihydroxyacetone phosphate
D-Glyceraldehyde-3-phosphate

Steps 2 (top) and 4 (bottom) of Glycolysis

With the pump thus primed, the pathway proceeds first to split the F1,6BP into two 3-carbon intermediates. Later the only oxidation step in the entire pathway occurs. In that reaction, **glyceraldehyde-3-phosphate** (G3P) is oxidized and a phosphate is added, creating **1,3-bisphosphoglycerate** (**1,3 BPG**).

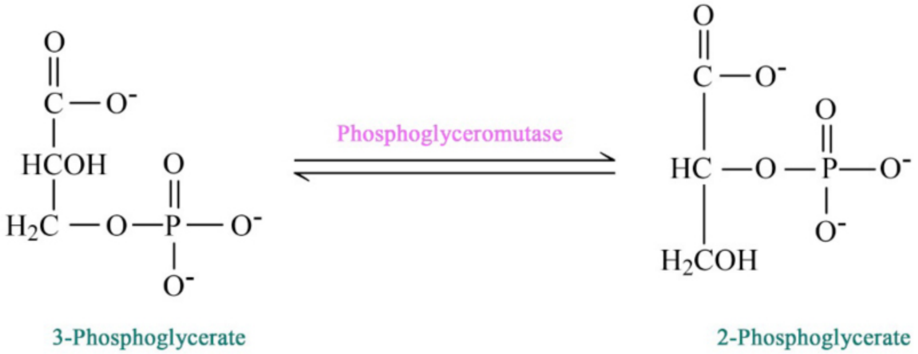
The addition of the phosphate sometimes conceals the oxidation that occurred. G3P was an aldehyde. 1,3 BGP is an acid esterified to a phosphate. The two phosphates in the tiny 1,3BPG molecule repel each other and give the molecule high energy. It uses this energy to phosphorylate ADP to make ATP.

Since there are two 1,3 BPGs produced for every glucose, the two ATP produced replenish the two ATPs used to start the cycle.

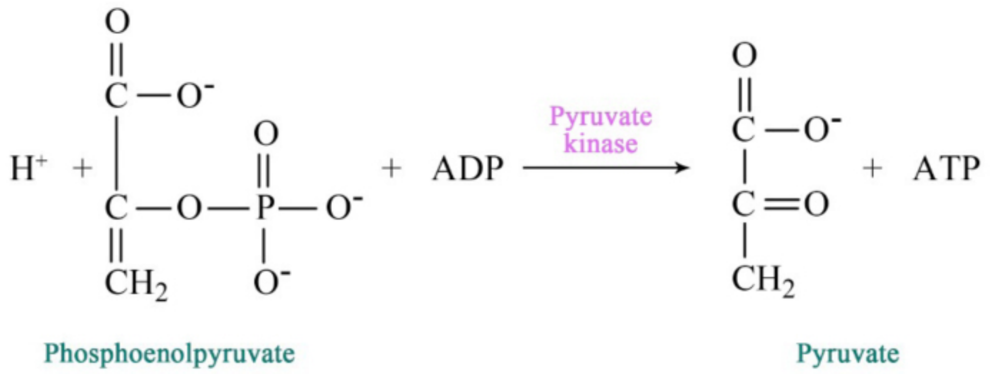

Glyceraldehyde-3-phosphate dehydrogenase
+ NAD+ + HO—P—O-
+ NADH+ + H+
Glyceraldehyde-3-phosphate
1,3-bisphosphoglycerate

Step 5 of Glycolysis

The synthesis of ATP directly from a metabolic reaction is known as **substrate level phosphorylation** (see HERE), though it is not a significant source of ATP. Glycolysis has two reactions during which substrate-level phosphorylation occurs.


Phosphoglyceromutase
3-Phosphoglycerate
2-Phosphoglycerate

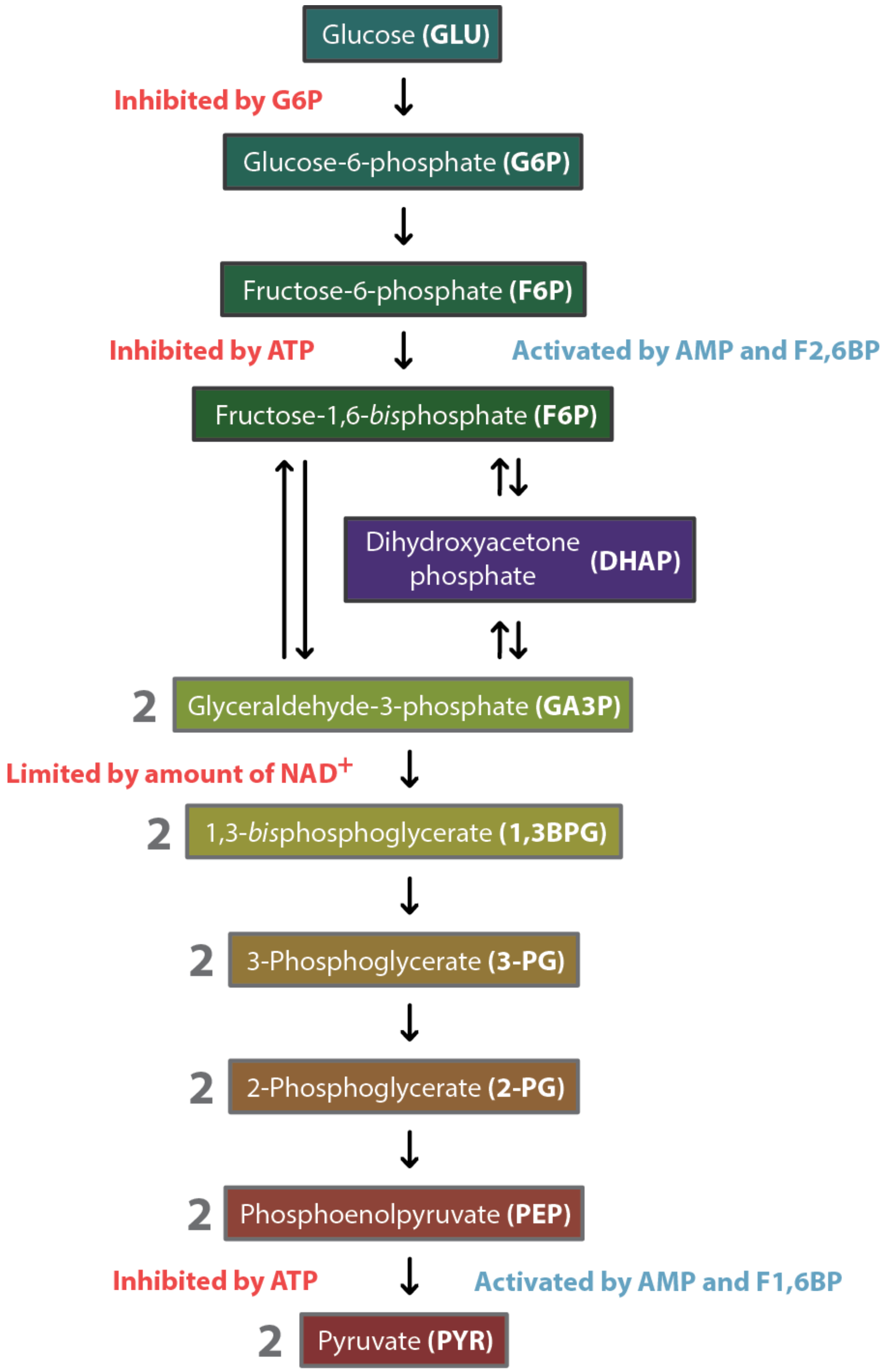
Step 8 of Glycolysis


H⁺
ADP
Pyruvate kinase
ATP
Phosphoenolpyruvate
Pyruvate

Step 10 of Glycolysis

The transfer of phosphate from 1,3BPG to ATP creates **3-phosphoglycerate** (**3-PG**). Conversion of 3-PG to **2-PG** occurs by an important mechanism. An intermediate in the reaction (catalyzed by **phosphoglycerate mutase**) is **2,3 BPG**. This intermediate, which is stable, is released with low frequency by the enzyme instead of being converted to 2-PG. 2,3BPG is important because it binds to hemoglobin and stimulates release of oxygen (see HERE). Thus, cells which are metabolizing glucose rapidly release more 2,3BPG and, as a result, stimulate release of more oxygen, supporting their needs.

2-PG is converted to **phosphoenolpyyruvate** (PEP) by removal of water, creating a very high energy intermediate. Conversion of PEP to pyruvate is the second substrate level phosphorylation of glycolysis, creating ATP. There is almost enough energy in PEP to stimulate production of a second ATP, but it is not used. Consequently, the energy is lost as heat. If you wonder why you get hot when you exercise, the reaction that converts PEP to


Glucose **(GLU)**
Inhibited by G6P
Glucose-6-phosphate **(G6P)**
Fructose-6-phosphate **(F6P)**
Inhibited by ATP
Activated by AMP and F2,6BP
Fructose-1,6-*bis*phosphate **(F6P)**
Dihydroxyacetone phosphate **(DHAP)**
2 Glyceraldehyde-3-phosphate **(GA3P)**
Limited by amount of NAD⁺
2 1,3-*bis*phosphoglycerate **(1,3BPG)**
2 3-Phosphoglycerate **(3-PG)**
2 2-Phosphoglycerate **(2-PG)**
2 Phosphoenolpyruvate **(PEP)**
Inhibited by ATP
Activated by AMP and F1,6BP
2 Pyruvate **(PYR)**

Glycolysis Regulation

pyruvate is a prime culprit.

Enzymes/Control

Control of glycolysis is unusual for a metabolic pathway, in that regulation occurs at three enzymatic points – **hexokinase** (Glucose <=>G6P), **phosphofructokinase** - **PFK** (F6P <=> F1,6BP), and **pyruvate kinase** (PEP <=> pyruvate).

Glycolysis is regulated in a reciprocal fashion compared to its corresponding anabolic pathway, gluconeogenesis. **Reciprocal regulation** occurs when the same molecule or treatment (**phosphorylation**, for example) has opposite effects on catabolic and anabolic pathways. Reciprocal regulation is important when anabolic and corresponding catabolic pathways are occurring in the same cellular location.

As an example, consider regulation of PFK. It is activated by several molecules, most importantly **fructose-2,6-bisphosphate** (**F2,6BP**). This molecule

The Sound of Glucose

To the tune of "*A Few of My Favorite Things*"

Aldehyde sugars are always aldoses and
If there's a ketone we call them ketoses
Some will form structures in circular rings
Saccharides do some incredible things

Onto a glucose we add a 'P' to it
ATP energy ought to renew it
Quick rearranging creates F6P
Without requiring input energy

At a high rate
Add a phosphate
With PFK
F1,6BP is made up this way
So we can run and play

Aldolase breaks it and then it releases
DHAP and a few G3Pieces
These both turn in to 1,3 BPG
Adding electrons onto NAD

Phosphate plus ADP makes ATP
While giving cells what they need - en-er-gy
Making triphosphate's a situa-shun
Of substrate level phosphoryla-shun

3-B-P-G
2-B-P-G
Lose a water
PEP gets a high energy state
Just to make py-ru-vate

So all the glucose gets broken and bent
If there's no oxygen cells must ferment
Pyruvate / lactate our cells hit the wall
Some lucky yeast get to make ethanol

This is the end of your glucose's song
Unless you goof up and get it all wrong
Break it, don't make it to yield ATP
You'll save your cells from fu-til-i-ty

Recorded by Tim Karplus
Lyrics by Kevin Ahern

has an inhibitory effect on the corresponding gluconeogenesis enzyme, **fructose-1,6-bisphosphatase** (**F1,6BPase**).

For cells a glucose cycling's cost
Is energy in reams
Four ATPs each time is lost
From breaking/making schemes

So use for metabolic heat
To make it warm inside your feet
Else it's of no utility
To practice such futility

You might wonder why pyruvate kinase, the last enzyme in the pathway, is regulated. The answer is simple. Pyruvate kinase catalyzes the most energetically rich reaction of glycolysis. The reaction is favored so strongly in the forward direction that cells must do a 'two-step' around it in the reverse direction when making glucose. In other words, it takes two enzymes, two reactions, and two triphosphates to go from pyruvate back to PEP in gluconeogenesis. When cells are needing to make glucose, they can't be sidetracked by having the PEP they have made in gluconeogenesis be converted directly back to pyruvate by pyruvate kinase. Consequently, pyruvate kinase is inhibited during gluconeogenesis, lest a "futile cycle" occur. (See HERE)

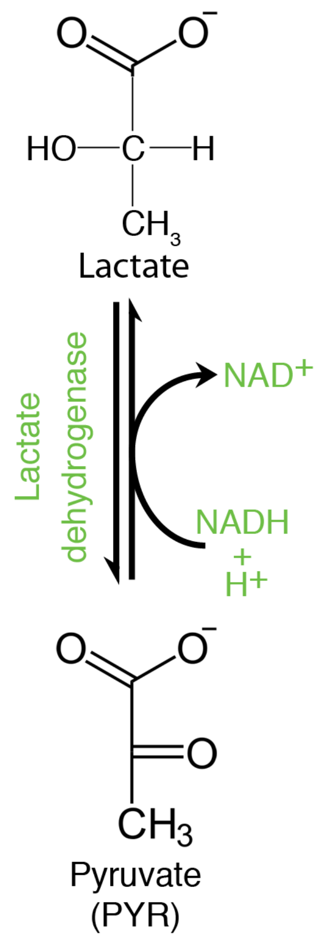
Another interesting control mechanism called **feedforward activation** involves pyruvate kinase. Pyruvate kinase is activated allosterically by F1,6BP. This molecule is a product of the PFK reaction and a substrate for the aldolase reaction. It should be noted that the aldolase reaction is energetically unfavorable (high + ΔG°'), thus allowing F1,6BP to accumulate. When this happens, some of the excess F1,6BP activates pyruvate kinase, which jump-starts the conversion of PEP to pyruvate. The resulting drop in PEP levels has the effect of "pulling" on the reactions preceding pyruvate kinase. As a consequence, the concentrations of G3P and DHAP fall, helping to move the aldolase reaction forward.

Pyruvate Metabolism

As noted, pyruvate produced in glycolysis can be oxidized to acetyl-CoA, which is itself oxidized in the citric acid cycle to carbon dioxide. That is not the only metabolic fate of pyruvate, though.

Pyruvate is a "starting" point for gluconeogenesis, being converted to oxaloacetate in the mitochondrion in the first step. Pyruvate in animals can also be reduced to **lactate** when oxygen is limiting. This reaction, which requires NADH produces NAD^+ and is critical for generating the latter molecule to keep the **glyceraldehyde-3-phosphate dehydrogenase** reaction of glycolysis going when there is no oxygen.

Oxygen is necessary for the electron transport system to operate and this, in turn, is what oxidizes NADH to NAD^+. In the absence of oxygen, thus, an alternative means of making NAD^+ is necessary, or else glycolysis will halt. Bacteria and yeast have NADH requiring reactions that regenerate NAD^+ while producing ethanol from pyruvate under anaerobic conditions, instead of lactic acid. Thus, **fermentation** of pyruvate is necessary to keep

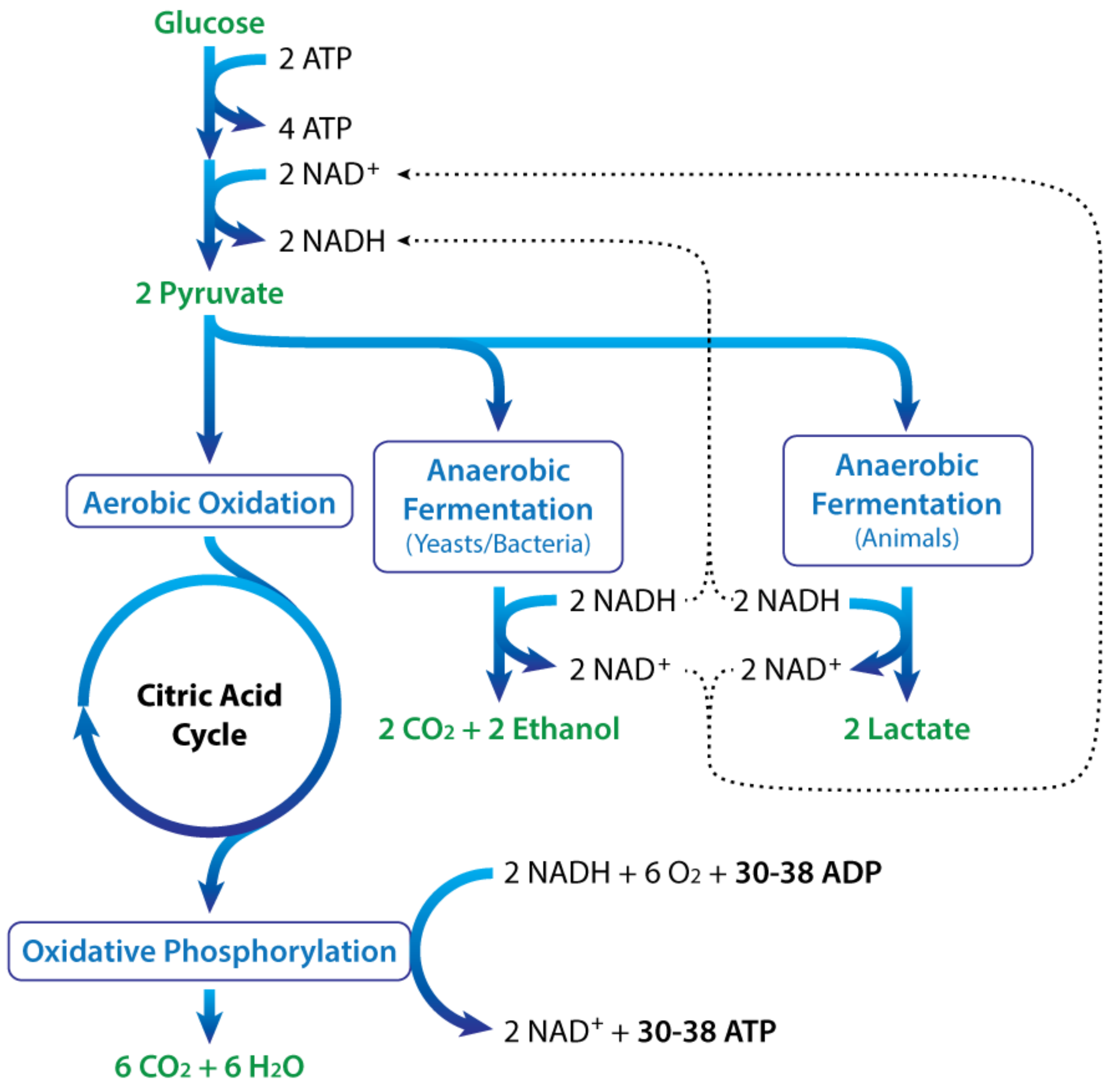

O
O−
HO—C—H
CH_3
Lactate
Lactate dehydrogenase
NAD+
NADH + H+
O
O−
O
CH_3
Pyruvate (PYR)

Pyruvate Reduction

glycolysis operating when oxygen is limiting. It is also for these reasons that brewing of beer (using yeast) involves depletion of oxygen and muscles low in oxygen produce lactic acid (animals).

Pyruvate is a precursor of alanine which can be easily synthesized by transfer of a nitrogen from an amine donor, such as glutamic acid. Pyruvate can also be converted into **oxaloacetate** by carboxylation in the process of gluconeogenesis (see below).

The enzymes involved in pyruvate metabolism include **pyruvate dehydrogenase** (makes acetyl-CoA), **lactate dehydrogenase** (makes lactate), **transaminases** (make alanine), and **pyruvate carboxylase** (makes oxaloacetate).


Glucose
2 ATP
4 ATP
2 NAD+
2 NADH
2 Pyruvate
Aerobic Oxidation
Anaerobic Fermentation (Yeasts/Bacteria)
Anaerobic Fermentation (Animals)
Citric Acid Cycle
2 NADH
2 NAD+
2 NADH
2 NAD+
2 CO2 + 2 Ethanol
2 Lactate
2 NADH + 6 O2 + 30-38 ADP
Oxidative Phosphorylation
2 NAD+ + 30-38 ATP
6 CO2 + 6 H2O

Metabolic Redox

Gluconeogenesis

The **anabolic** counterpart to glycolysis is gluconeogenesis, which occurs mostly in the cells of the liver and kidney. In seven of the eleven reactions of gluconeogenesis (starting from pyruvate), the same enzymes are used as in glycolysis, but the reaction directions are reversed. Notably, the ΔG values of these reactions in the cell are typically near zero, meaning their direction can be readily controlled by changing substrate and product concentrations.

Click HERE and HERE to see Kevin's YouTube lectures on Gluconeogenesis

The three regulated enzymes of glycolysis all catalyze reactions whose **ΔG** values are not close to

Gluconeogenesis

To the tune of “*Supercalifragelisticexpialidocious*”

When cells have lots of ATP and NADH too
They strive to store this energy as sugar yes they do
Inside of mitochondria they start with pyruvate
(slow) Carboxylating it to make oxaloacetate

Oh gluconeogenesis is so exhilarating
Memorizing it can really be exasperating
Liver cells require it so there’s no need for debating
Gluconeogenesis is so exhilarating

Oh, glucose, glucose come to be
Glucose, glucose come to be

Oxaloacetate has got to turn to PEP
Employing energy that comes from breaking GTP
From there it goes to make a couple phosphoglycerates
(slow) Exploiting ee-nolase and mutase’ catalytic traits

Oh gluconeogenesis is liver’s specialty
Producing sugar for the body most admirably
Six ATPs per glucose is the needed energy*
Gluconeogenesis is liver’s specialty

Oh glucose, glucose joy to me
Glucose, glucose joy to me

Converting phosphoglycerate to 1,3BPG
Requires a phosphate that includes A-T-P energy
Reduction with electrons gives us all an N-A-D
(slow) And G3P’s isomerized to make D-H-A-P

Oh gluconeogenesis is anabolic bliss
Reversing seven mechanisms of glycolysis
To do well on the final students have to learn all this
Gluconeogenesis is anabolic bliss

Oh, glucose, glucose factory
Glucose, glucose factory

The aldolase reaction puts together pieces so
A fructose molecule is made with two phosphates in tow
And one of these gets cleaved off by a fructose phosphatase
(slow) Unless F2,6BP's acting blocking path-a-ways

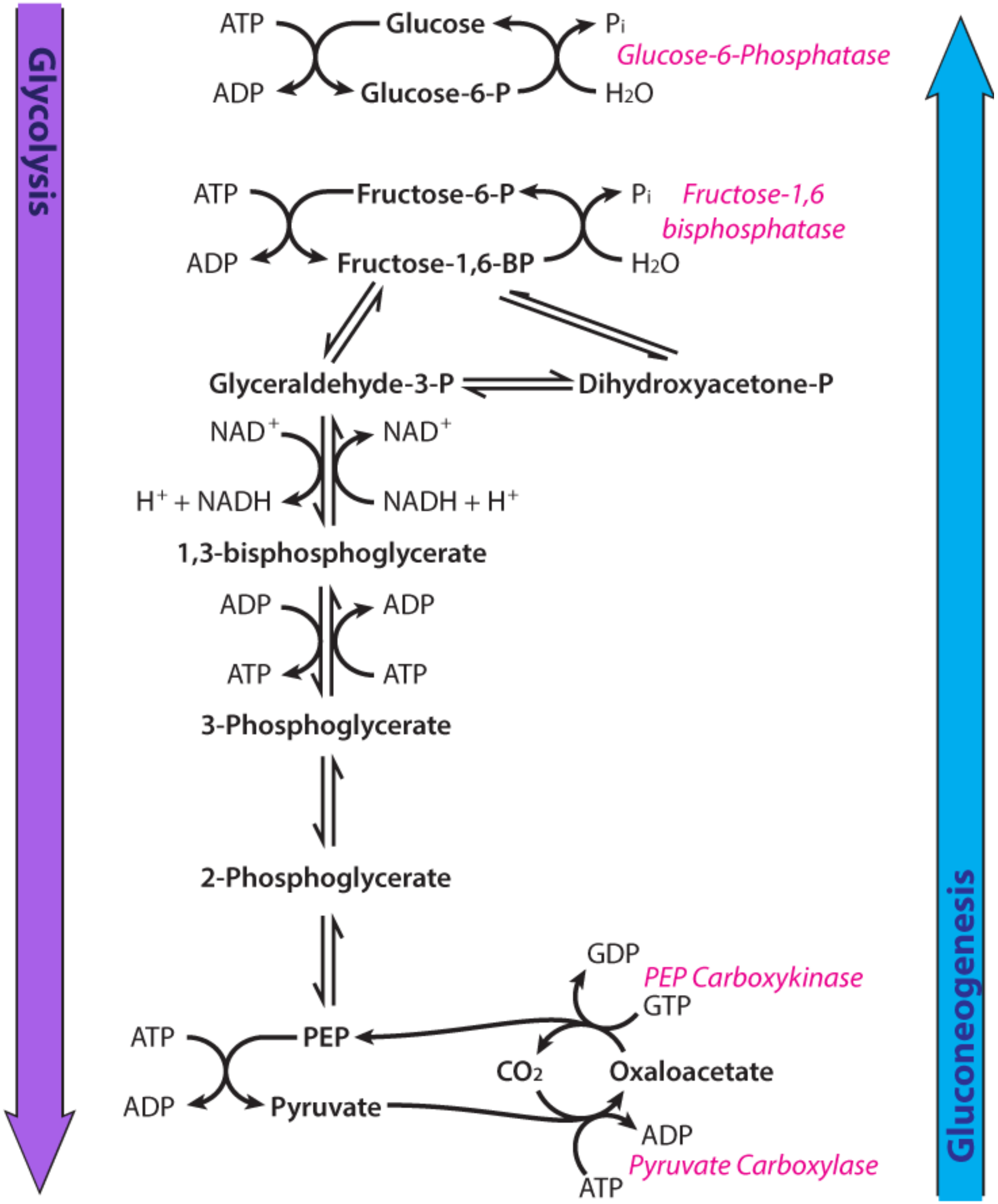
Oh gluconeogenesis a pathway to revere
That makes a ton of glucose when it kicks into high gear
The cell's a masterminding metabolic engineer
Gluconeogenesis a pathway to revere

Oh glucose, glucose jubilee
Glucose, glucose jubilee

From F6P to G6P, that is the final phase
The enzyme catalyzing it is an isomerase
Then G6P drops phosphate and a glucose it becomes
(slow) Inside the tiny endoplasmic-al reticulums

Oh gluconeogenesis is not so very hard
I know that on the final we will not be caught off guard
Cuz our professor lets us use a filled out index card
Gluconeogenesis is not so very hard

Recorded by Tim Karplus
Lyrics by Kevin Ahern


Glycolysis
ATP
ADP
Glucose
Glucose-6-P
Pi
H2O
Glucose-6-Phosphatase
ATP
ADP
Fructose-6-P
Fructose-1,6-BP
Pi
H2O
Fructose-1,6 bisphosphatase
Glyceraldehyde-3-P
Dihydroxyacetone-P
NAD+
H+ + NADH
NAD+
NADH + H+
1,3-bisphosphoglycerate
ADP
ATP
ADP
ATP
3-Phosphoglycerate
2-Phosphoglycerate
GDP
PEP Carboxykinase
GTP
ATP
ADP
PEP
Pyruvate
CO2
Oxaloacetate
ADP
Pyruvate Carboxylase
ATP
Gluconeogenesis

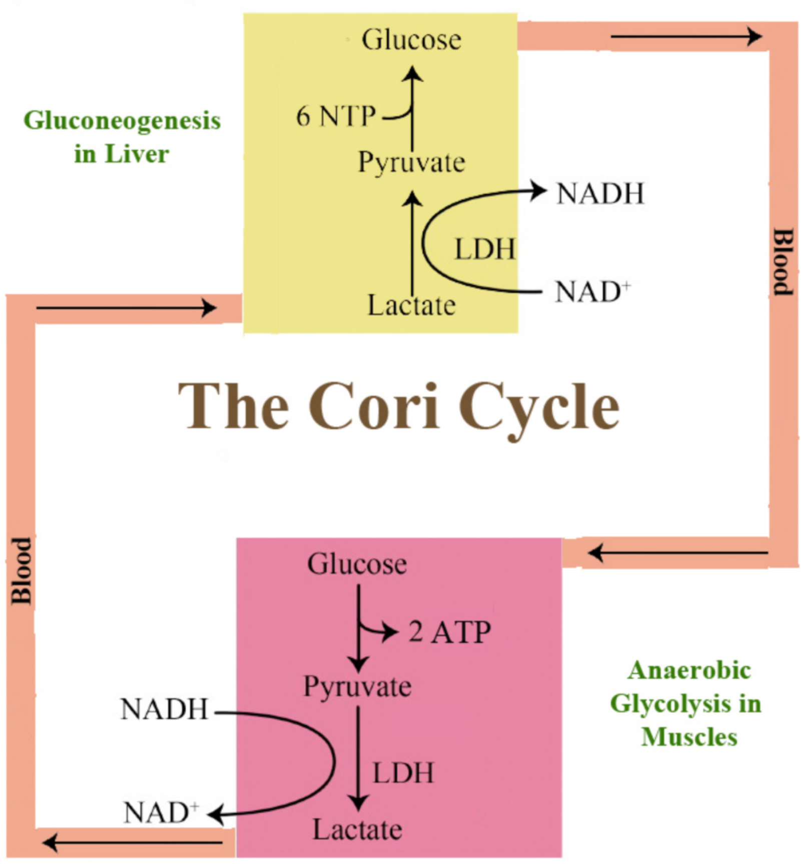
zero, making manipulation of reaction direction non-trivial. Consequently, cells employ "work-around" reactions catalyzed by four different enzymes to favor gluconeogenesis, when appropriate.

Directional velocity
Inverts with reciprocity
If glycolysis is flowing
Glucose synthesis awaits
But when the latter is a-going
Sugar breakdown then abate

Two of the enzymes (pyruvate carboxylase and PEP carboxykinase - **PEPCK**) catalyze reactions that bypass pyruvate kinase. F1,6BPase bypasses PFK and **G6Pase** bypasses hexokinase. Notably, pyruvate carboxylase and G6Pase are found in the mitochondria and endoplasmic reticulum, respectively, whereas the other two are found in the cytoplasm along with all of the enzymes of glycolysis. As a result, all of glycolysis and most of gluconeogenesis occurs in the cytoplasm. Controlling these pathways then becomes of critical importance because cells generally need to minimize the extent to which paired anabolic and catabolic pathways are occurring simultaneously, lest they waste energy and make no tangible product except heat. The mechanisms of controlling these pathways work, in some ways, in opposite fashions, called reciprocal regulation (see above).

Besides reciprocal regulation, other mechanisms help control gluconeogenesis. First, PEPCK is controlled largely at the level of synthesis. Overexpression of PEPCK (stimulated by glucagon, glucocorticoids, and cAMP and inhibited by insulin) causes symptoms of diabetes. Pyruvate carboxylase is sequestered in the mitochondrion and is sensitive to acetyl-CoA, which is an allosteric activator. Acetyl-CoA concentrations increase as the citric acid cycle activity decreases. **Glucose-6-phosphatase** is present in low concentrations in many tissues, but is found most abundantly and importantly in the major gluconeogenic organs – the liver and kidney cortex.

Cori Cycle



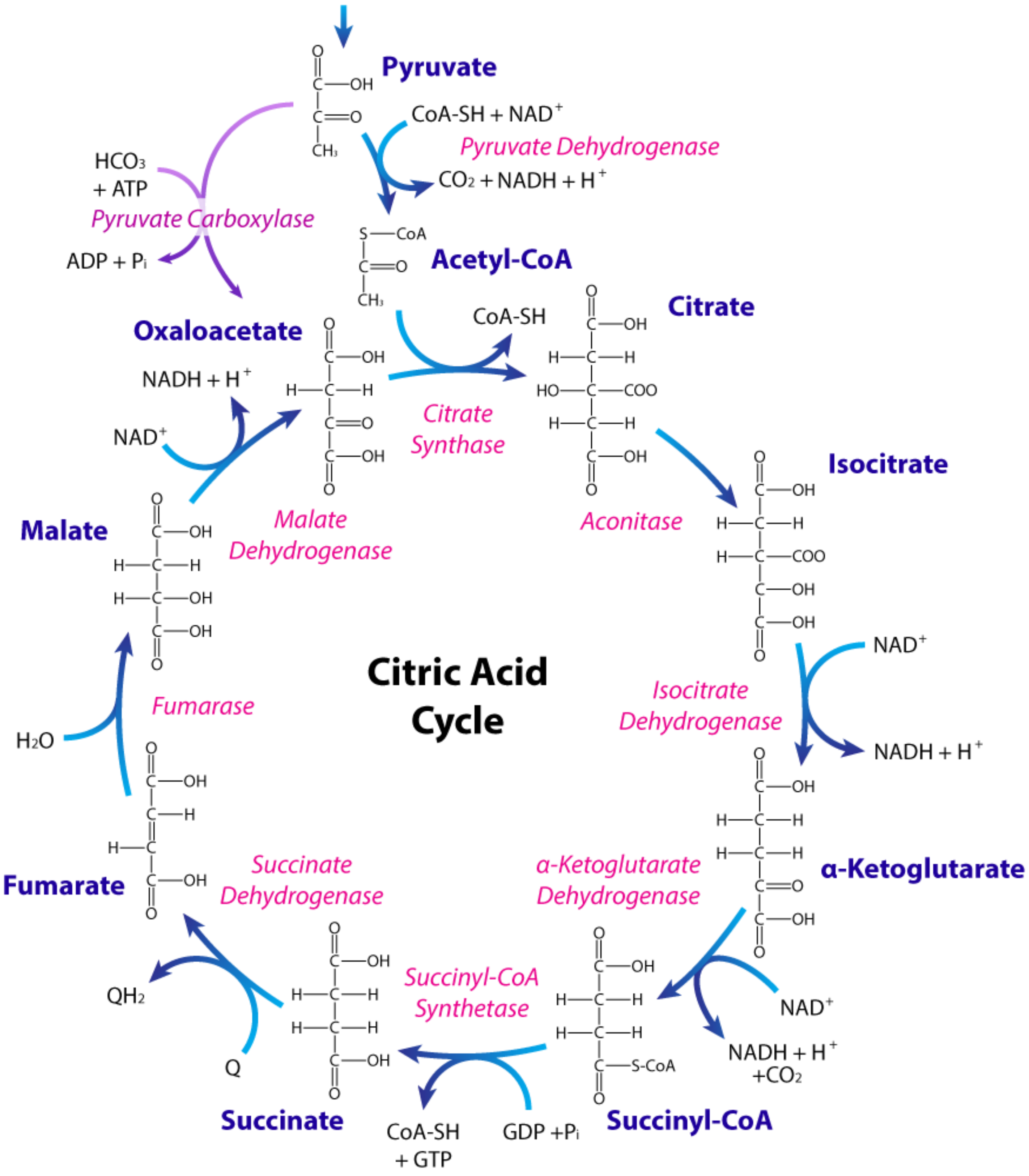
With respect to energy, the liver and muscles act complementarily. The liver is the major organ in the body for the synthesis of glucose. Muscles are major users of ATP. Actively exercising muscles generate lactate as a result of running glycolysis faster than the blood can deliver oxygen during periods of heavy exercise. As a consequence, the muscles go anaerobic and produce lactate. This lactate is of no use to muscle cells, so they dump it into the blood. Lactate travels in the blood to the liver, which takes it up and reoxidizes it back to pyruvate, catalyzed by the enzyme lactate dehydrogenase. Pyruvate in the liver is then converted to glucose by gluconeogenesis. The glucose thus made by the liver is dumped into the bloodstream where it is taken up by muscles and used for energy, completing a very important intercellular pathway known as the Cori cycle.

Citric Acid Cycle

The primary catabolic pathway in the body is the **citric acid cycle** because it is here that oxidation to carbon dioxide occurs for breakdown products of the cell's major building blocks - sugars, fatty acids, amino acids. The pathway is cyclic (next page) and thus, doesn't really have a starting or ending point. All of the reactions occur in the mitochondrion, though one enzyme is embedded in the organelle's membrane. As needs change, cells may use a subset of the reactions of the cycle to produce a desired molecule rather than to run the entire cycle (see below for examples).

Click HERE and HERE for Kevin's YouTube lectures on the Citric Acid and Glyoxylate Cycles

Focusing on the pathway itself, the traditional point to start discussion is addition


Pyruvate
CoA-SH + NAD$^+$
Pyruvate Dehydrogenase
CO$_2$ + NADH + H$^+$
HCO$_3$
+ ATP
Pyruvate Carboxylase
ADP + P$_i$
Acetyl-CoA
Oxaloacetate
CoA-SH
Citrate
Citrate Synthase
NADH + H$^+$
NAD$^+$
Malate Dehydrogenase
Malate
Aconitase
Isocitrate
Citric Acid Cycle
Fumarase
NAD$^+$
Isocitrate Dehydrogenase
NADH + H$^+$
H$_2$O
α-Ketoglutarate
Fumarate
Succinate Dehydrogenase
α-Ketoglutarate Dehydrogenase
Succinyl-CoA Synthetase
QH$_2$
NAD$^+$
NADH + H$^+$
+CO$_2$
Q
Succinate
CoA-SH
+ GTP
GDP +P$_i$
Succinyl-CoA

The Citric Acid Cycle Reactions

of acetyl-CoA to oxaloacetate (OAA) to form citrate. Acetyl-CoA for the pathway can come from a variety of sources. They include pyruvate oxidation (from glycolysis and amino acid metabolism), fatty acid oxidation, and amino acid metabolism. The reaction joining it to OAA is catalyzed by citrate synthase and the ΔG°' is fairly negative. This, in turn, helps to "pull" the reaction preceding it in the cycle (catalyzed by malate dehydrogenase).

In the next reaction, citrate is isomerized to **isocitrate** by action of the enzyme called **aconitase**. Isocitrate is a branch point in plants and bacteria for the **glyoxylate cycle**. Oxidative decarboxylation of isocitrate by isocitrate dehydrogenase produces the first NADH and yields **alpha-ketoglutarate**. This five carbon intermediate is a branch point for synthesis of glutamate. In addition, glutamate can also be made easily into this citric acid cycle intermediate. Decarboxylation of alpha-ketoglutarate yields **succinyl-CoA** and is catalyzed by **alpha-ketoglutarate dehydrogenas**e. This enzyme is structurally very similar to pyruvate dehydrogenase and employs the same five coenzymes – **NAD**, FAD, **CoASH**, **TPP**, and **lipoic acid**.

The remainder of the citric acid cycle involves

I love my citrate synthase
It really is first rate
Adds O-A-A to Ac-Co-A
Producing a citrate

Aconitase is picky
Binds substrates specially
Creating isocitrate
Which has no symmetry

Then CO_2 gets lost from it
Released in the next phase
The secret weapon - Isocitrate
Dehydrogenase

The alpha K–D-H is next
It gets my admiration
For clipping CO_2 in one more
Decarboxylation

Succ-CoA synthetase steps up
Reacting most absurd
It's named for a catalysis
That really goes backward

Suc -CIN-ate de-hyd-ROG-en-ase
Pulls H from succinate
Creating $FADH_2$
As well as fumarate

The fumarate gains water
OH-configured L
The fumarase's product?
Some malate for the cell

With the last oxidation
Malate de-hyd-ROG-en-ase
Expels its two creations
N-A-D-H / O-A-A

conversion of the four carbon succinyl-CoA into oxaloacetate. Succinyl-CoA is a branch point for the synthesis of heme. Succinyl-CoA is converted to **succinate** in a reaction catalyzed by **succinyl-CoA synthetase** (named for the reverse reaction) and a GTP is produced, as well – the only substrate level phosphorylation in the cycle. The energy for the synthesis of the GTP comes from hydrolysis of the high energy **thioester** bond between succinate and the CoA. Evidence for the high energy of a thioester bond is also evident in the citrate synthase reaction, which is also very energetically favorable. Succinate is also produced by metabolism of odd-chain fatty acids (see below).

Oxidation of succinate occurs in the next step, catalyzed by **succinate dehydrogenase**. This interesting enzyme both catalyzes this reaction and participates in the electron transport system, funneling electrons from the $FADH_2$ it gains in the reaction to **coenzyme Q**. The product of the reaction, **fumarate** gains a water across its *trans* double bond in the next reaction, catalyzed by **fumarase** to form **malate**. Fumarate is also a byproduct of nucleotide metabolism and of the **urea cycle**. Malate is important also for transporting electrons across membranes in the **malate aspartate shuttle** and in ferrying carbon dioxide in **C_4 plants**.

Conversion of malate to OAA is a rare biological oxidation that has a $\Delta G^{\circ\prime}$ with a positive value. The reaction product includes NADH and the reaction is 'pulled' by the energetically favorable conversion of OAA to citrate in what was described above as the first reaction of the cycle. OAA intersects other important pathways, including amino acid metabolism (readily converted to

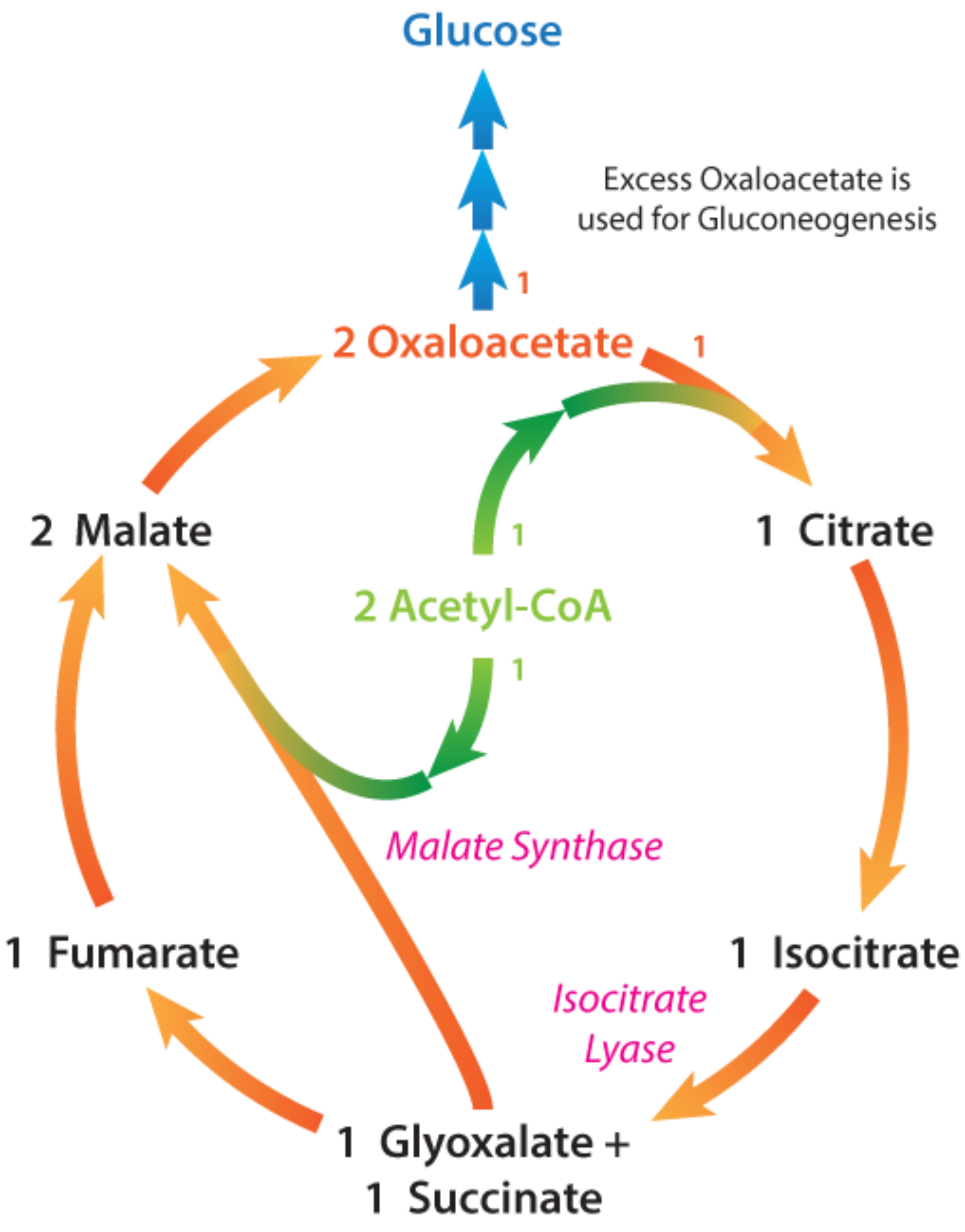
I'm thinking I could lose some weight
If I could make glyoxylate
Combined with acetyl-CoA
Malate would then form OAA
The excess OAA in turn
Would give more glucose to be burned
Converting fat to glucose, see
Expends it glycolytically

aspartic acid), **transamination** (nitrogen movement) and gluconeogenesis.

Glyoxylate Pathway

A pathway related to the Citric Acid Cycle (CAC) is the **glyoxylate pathway** (right). This pathway, which overlaps all of the non-decarboxylation reactions of the CAC does not operate in animals, because they lack two enzymes necessary for the pathway – **isocitrate lyase** and **malate synthase**. Isocitrate lyase catalyzes the conversion of isocitrate into succinate and glyoxylate. Because of this, all six carbons of the CAC survive and do not end up as carbon dioxide.

Succinate continues through the remaining reactions of the CAC to produce oxaloacetate. Glyoxylate combines with another acetyl-CoA (one acetyl-CoA was used to start the cycle) to create malate (catalyzed by malate synthase). Malate can, in turn, be oxidized to oxaloacetate.


Glucose
Excess Oxaloacetate is used for Gluconeogenesis
1
2 Oxaloacetate
1
2 Malate
1 Citrate
1
2 Acetyl-CoA
1
Malate Synthase
1 Fumarate
1 Isocitrate
Isocitrate Lyase
1 Glyoxalate +
1 Succinate

The Glyoxylate Cycle

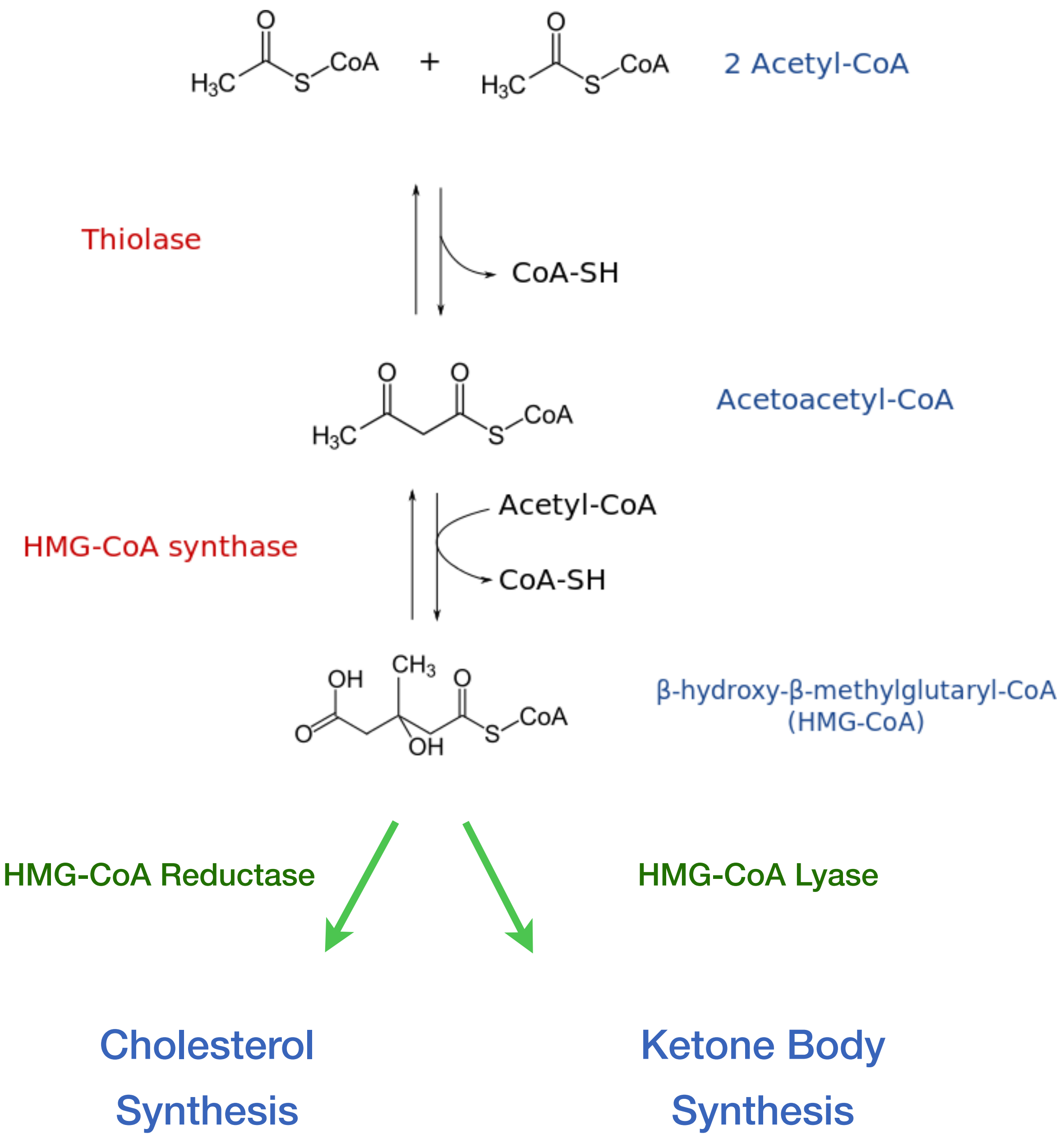
It is at this point that the pathway's contrast with the CAC is apparent. After one turn of the CAC, a single oxaloacetate is produced and it balances the single one used in the first reaction of the cycle. Thus, in the CAC, no net production of oxaloacetate is realized. By contrast, at the end of a turn of the glyoxylate cycle, two oxaloacetates are produced, starting with one. The extra oxaloacetate can then be used to make other molecules, including glucose in gluconeogenesis.

Because animals do not run the glyoxylate cycle, they cannot produce glucose from acetyl-CoA in net amounts, but plants and bacteria can. As a result, these organisms can turn acetyl-CoA from fat into glucose, while animals can't. Bypassing the decarboxylations (and substrate level phosphorylation) has its costs, however. Each turn of the glyoxylate cycle produces one FADH$_2$

and one NADH instead of the three NADHs, one $FADH_2$, and one GTP made in each turn of the CAC.

Acetyl-CoA Metabolism

Acetyl-CoA is one of the most “connected” metabolites in biochemistry, appearing in fatty acid oxidation/ reduction, pyruvate oxidation, the citric acid cycle, amino acid anabolism/catabolism, ketone body metabolism, steroid/bile acid synthesis, and (by extension from fatty acid metabolism) prostaglandin synthesis. Most of these pathways will be dealt with separately. Here we will cover the last three.


2 Acetyl-CoA
Thiolase
CoA-SH
Acetoacetyl-CoA
HMG-CoA synthase
Acetyl-CoA
CoA-SH
β-hydroxy-β-methylglutaryl-CoA
(HMG-CoA)
HMG-CoA Reductase
HMG-CoA Lyase
Cholesterol Synthesis
Ketone Body Synthesis

The pathways for ketone body synthesis and cholesterol biosynthesis overlap at the beginning. Each of these starts by combining two acetyl-CoAs together to make acetoacetyl-CoA. Not coincidentally, that is the next to last product of oxidation of fatty acids with even numbers of carbons (see below). In fact, the enzyme that catalyzes the joining is the same as the one that catalyzes its breakage in fatty acid oxidation – **thiolase**. Thus, these pathways start by reversing the last step of the last round of fatty acid oxidation. Both pathways also include addition of two more carbons from a third acetyl-CoA to form **Hydroxy-Methyl-Glutaryl-CoA**, or **HMG-CoA**, as it is more commonly known. It is at this point that the two pathways diverge.

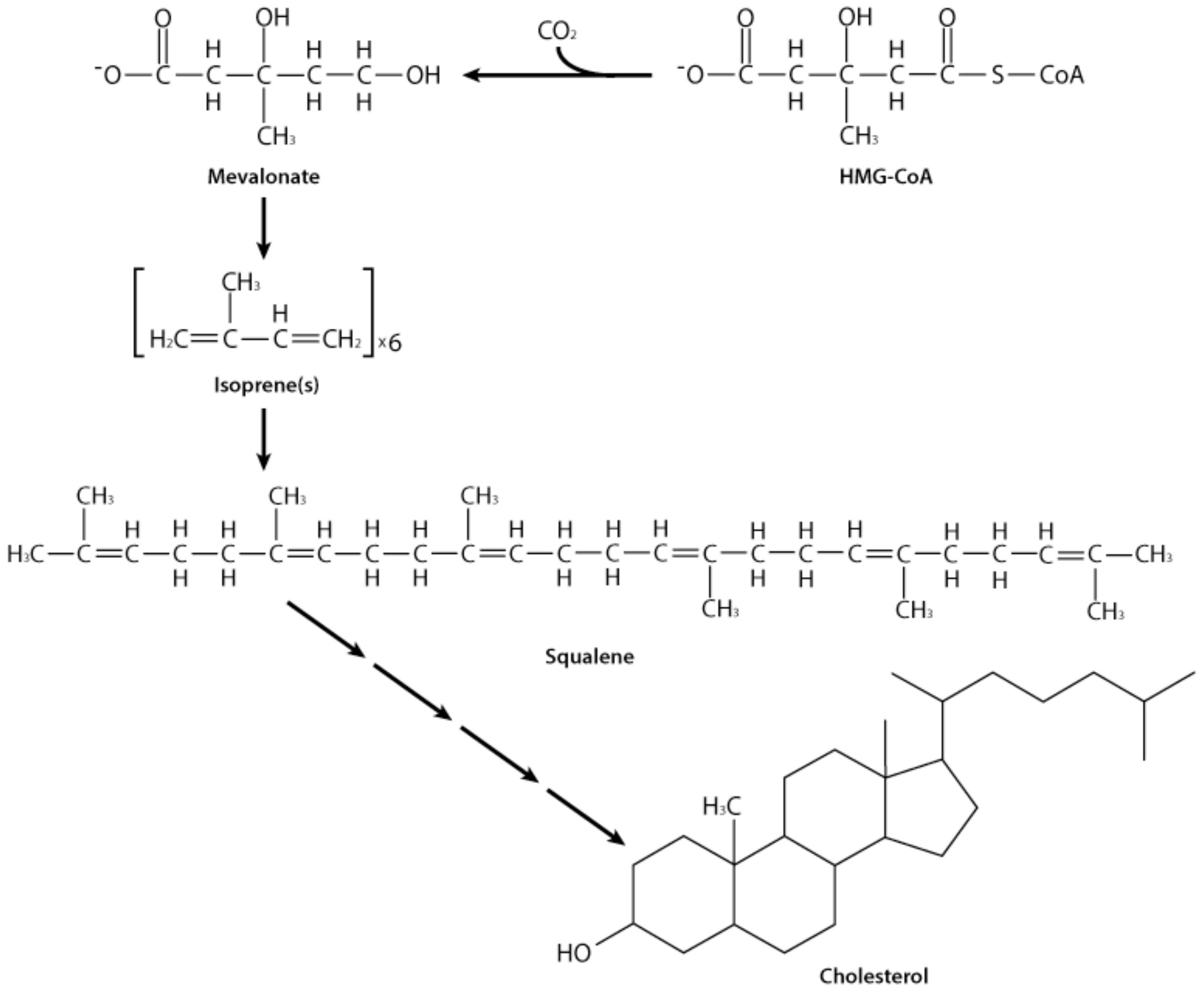
Cholesterol Metabolism

The cholesterol biosynthesis pathway is a long one and it requires significant amounts of reductive and ATP energy, which is why it is included here. Cholesterol has important roles in the body in membranes. It as also a precursor of **steroid hormones** and **bile acids** and its immediate metabolic precursor, **7-dehydrocholesterol**, is a precursor of **Vitamin D**. The pathway leading to cholesterol is known as the **isoprenoid pathway** and branches of it lead to other molecules including other fat-soluble vitamins.

From HMG-CoA, the enzyme HMG-CoA reductase catalyzes the formation of **mevalonate**. The reaction requires NADPH and results in release of coenzyme A and appears to be one of the most important regulatory steps in the synthesis pathway. The enzyme is regulated both by feedback inhibition (cholesterol inhibits it) and by covalent modification (phosphorylation inhibits it). The enzyme's synthesis is also regulated transcriptionally. When cholesterol levels fall, transcription of the gene increases.

See Kevin's YouTube lectures on Steroid and Lipid Metabolism and Lipid Movement in the Body HERE and HERE

Mevalonate gets phosphorylated twice and then decarboxylated to yield the five carbon intermediate known as **isopentenyl-pyrophosphate** (IPP). IPP is readily converted to **dimethylallylpyrophosphate** (DMAPP).



The Pathway to Cholesterol

These two five carbon compounds, also called isoprenes, are the building blocks for the synthesis of cholesterol and related compounds. This pathway is known as the isoprenoid pathway. It proceeds in the direction of cholesterol starting with the joining of IPP and DMAPP to form **geranyl-pyrophosphate**. Geranyl-pyrophosphate combines with another IPP to make **farnesyl-pyrophosphate**, a 15-carbon compound. Two farnesyl-pyrophosphates join to create the 30-carbon

To Make a Cholesterol

To the tune of “*When Johnny Comes Marching Home*”

Some things that you can build with acetyl-CoAs
Are joined together partly thanks to thiolase
They come together 1-2-3
Six carbons known as H-M-G
And you’re on your way
To make a cholesterol

To synthesize a mevalonate in the cell
Requires reducing HMG-CoA, as well
The enzyme is a RE-ductase
Controlled in allosteric ways
When the cell's impelled
To make a cholesterol.

The mevalonate made in metabolic schemes
Gets decarboxylated down to isoprenes
They’re linked together willy-nil
To build a PP-geranyl
In the cells’ routinesTo make a cholesterol

A single step links farnesyls but that’s not all
The squalene rearranges to lanosterol
From that there’s nineteen steps to go
Before the sterol’s apropos
Which you must recall
To make a cholesterol

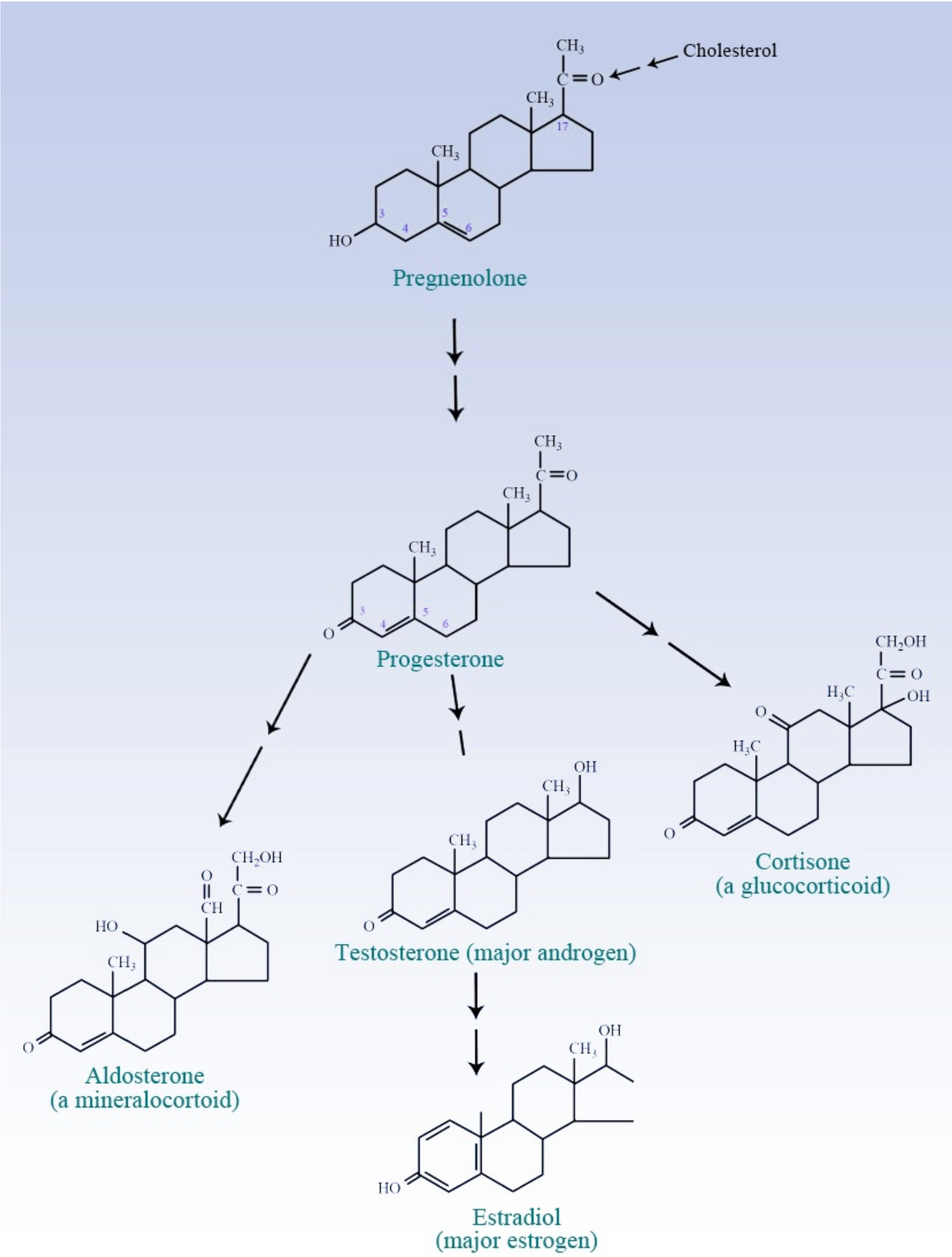
The regulation of the scheme’s complex in ways
Inhibited by feedback of the RE-duc-tase
And statins mimic so they say
The look of HMG-CoA
So we sing their praise
And not make cholesterol

Recorded by David Simmons
Lyrics by Kevin Ahern

compound known as **squalene**. Squalene, in a complicated rearrangement involving reduction and molecular oxygen forms a cyclic intermediate known as lanosterol that resembles cholesterol. Conversion of lanosterol to cholesterol is a lengthy process involving 19 steps that occur in the endoplasmic reticulum.

Branching from cholesterol, one can form Vitamin D or the steroid hormones, which include the progestagens, androgens, estrogens, mineralocorticoids, and the glucocorticoids (pictured on the previous page). The branch molecule for all of these is the cholesterol metabolite (and progestagen) known as pregnenalone. The progestagens are precursors of all of the other classes.

The estrogens are derived from the androgens in an interesting reaction that required formation of an aromatic ring. The enzyme catalyzing this reaction is known as an aromatase and it is of medical significance. The growth of some tumors is stimulated by estrogens, so aromatase inhibitors are prescribed to prevent the formation of estrogens and slow tumor growth.

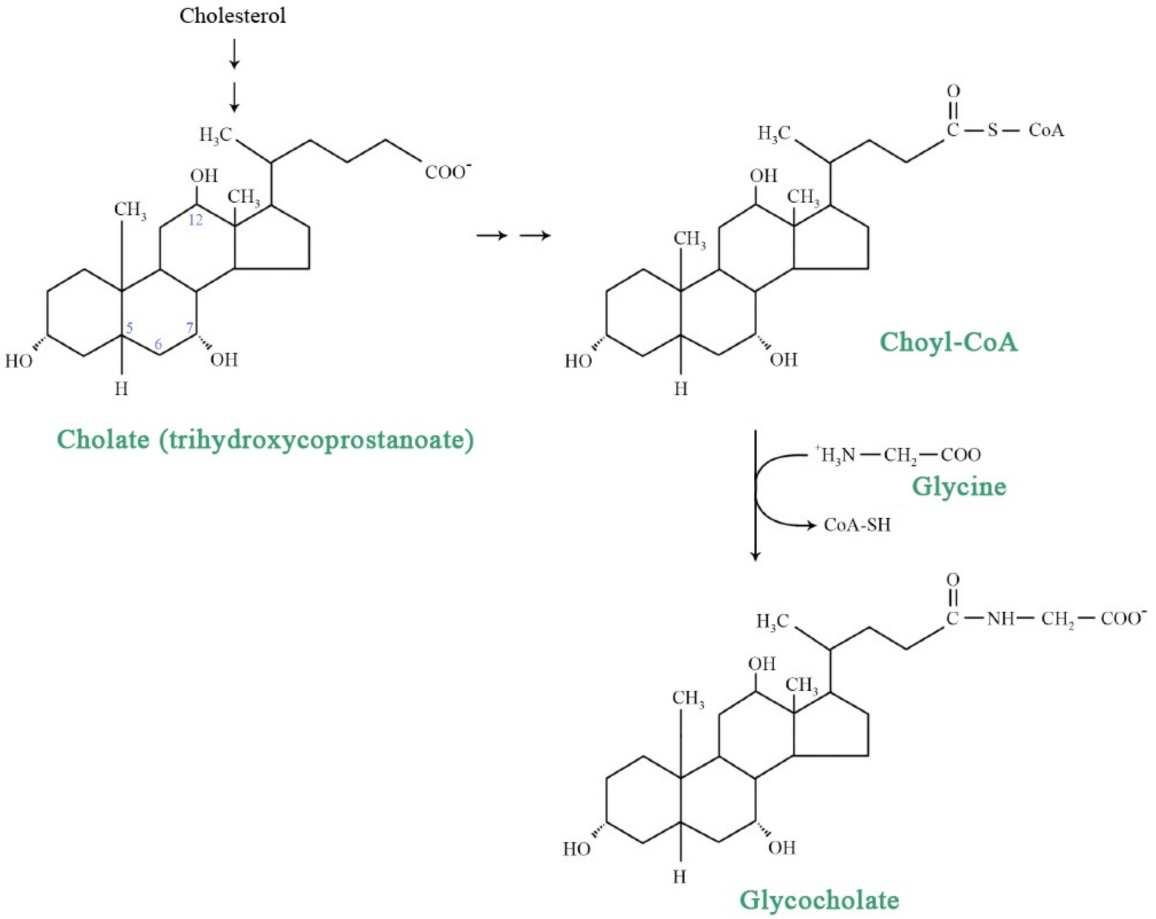

Cholesterol
Pregnenolone
Progesterone
Aldosterone
(a mineralocortoid)
Testosterone (major androgen)
Cortisone
(a glucocorticoid)
Estradiol
(major estrogen)

Steroid Hormone Synthesis

It is worth noting that synthesis of other fat soluble vitamins and **chlorophyll** also branches from the isoprenoid synthesis pathway at geranylpyrophosphate. Joining of two geranylgeranylpyrophosphates occurs in plants and bacteria and leads to synthesis of **lycopene**, which, in turn is a precursor of **beta-carotene**, the final precursor of **Vitamin A**. Vitamins E and K, as well as chlorophyll are all also synthesized from geranylgeranylpyrophosphate.

Bile Acid Metabolism

Another pathway from cholesterol leads to the polar bile acids, which are important for the solubilization of fat during digestion. Converting the very non-polar cholesterol to a bile acid involves


Cholesterol
Cholate (trihydroxycoprostanoate)
Choyl-CoA
Glycine
CoA-SH
Glycocholate

Bile Salts

oxidation of the terminal carbon on the side chain off the rings. Other alterations to increase the polarity of these compounds include hydroxylation of the rings and linkage to other polar compounds.

Common bile acids include **cholic acid**, **chenodeoxycholic acid**, **glycocholic acid**, **taurocholic acid**, and **deoxycholic acid**. Another important fact about bile acids is that their synthesis reduces the amount of cholesterol available and promotes uptake of LDLs by the liver. Normally bile acids are recycled efficiently resulting in limited reduction of cholesterol levels. However, inhibitors of the recycling promote reduction of cholesterol levels.

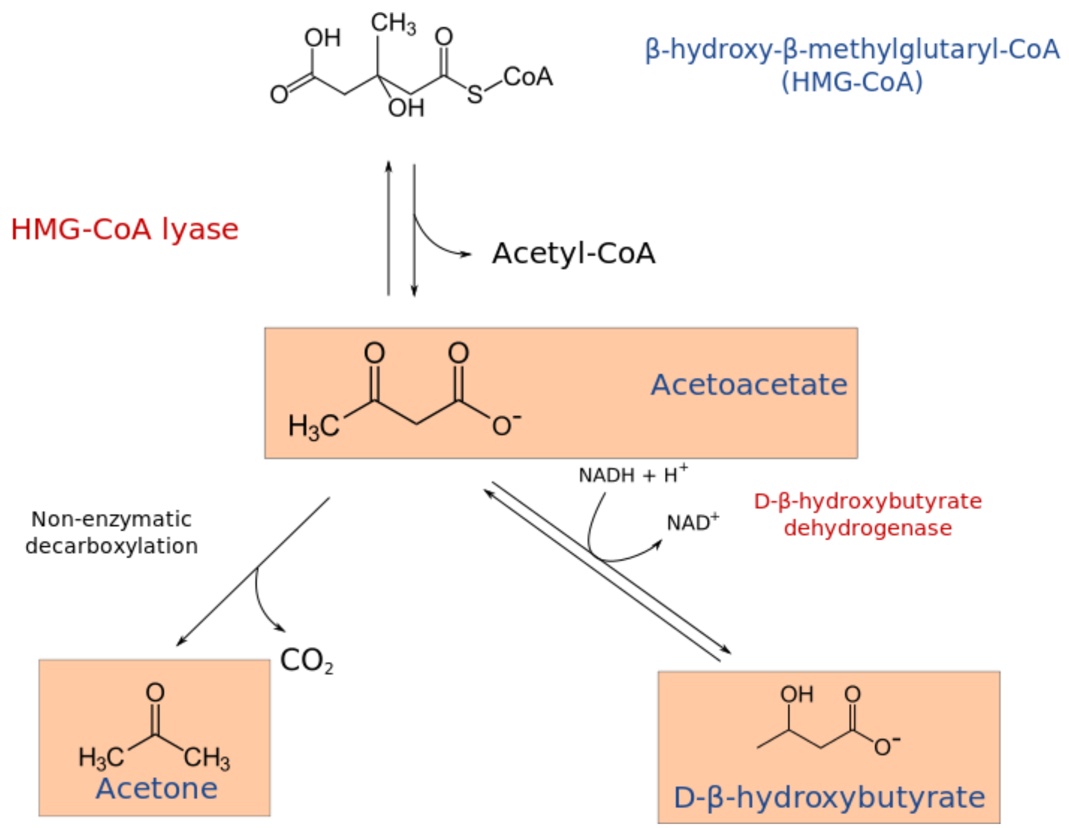
Ketone Body Metabolism

In ketone body synthesis, an acetyl-CoA is split off from HMG-CoA, yielding acetoacetate, a four carbon ketone body that is somewhat unstable, chemically. It will decarboxylate spontaneously to some extent to yield acetone.

Ketone bodies are made when the blood levels of glucose fall very low. Ketone bodies can be converted to acetyl-CoA, which can be used for ATP synthesis via the citric acid cycle. People who are very hypoglycemic (including some diabetics) will produce ketone bodies and these are often first detected by the smell of acetone on their breath.

Acetone is of virtually no use for energy production since it is not readily converted to acetyl-CoA.

See Kevin's lecture on
Ketone Bodies HERE

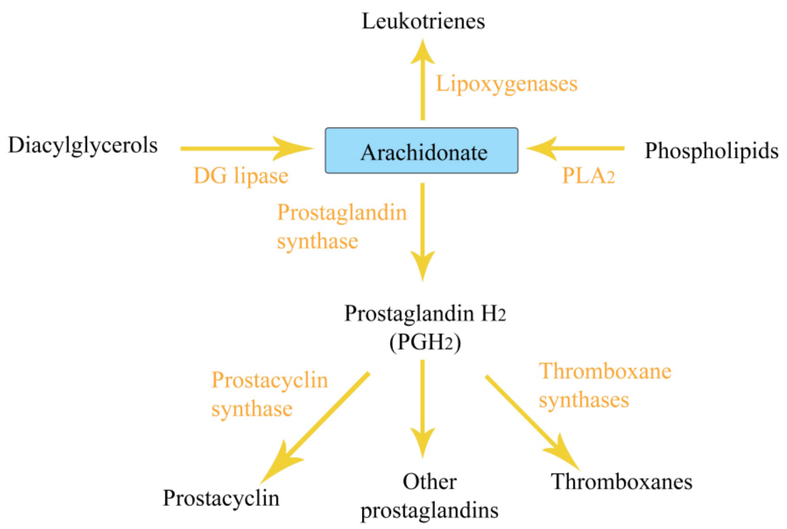

β-hydroxy-β-methylglutaryl-CoA
(HMG-CoA)
HMG-CoA lyase
Acetyl-CoA
Acetoacetate
Non-enzymatic decarboxylation
CO₂
NADH + H⁺
NAD⁺
D-β-hydroxybutyrate dehydrogenase
Acetone
D-β-hydroxybutyrate

Ketone Body Reactions

Consequently, cells convert acetoacetate into beta-hydroxybutyrate, which is more chemically stable. Though technically not a ketone, beta-hydroxybutyrate is frequently referred to as a ketone body. Upon arrival at a target cell, it can be oxidized back to acetoacetate with conversion to acetyl-CoA. Both **acetoacetate** and **beta-hydroxybutyrate** can cross the blood-brain barrier and provide important energy for the brain when glucose is limiting.

Prostaglandin Synthesis

The pathway for making **prostaglandins** is an extension of the fatty acid synthesis pathway


Leukotrienes
Lipoxygenases
Diacylglycerols
DG lipase
Arachidonate
Phospholipids
PLA2
Prostaglandin synthase
Prostaglandin H2 (PGH2)
Prostacyclin synthase
Thromboxane synthases
Prostacyclin
Other prostaglandins
Thromboxanes

Prostaglandin Metabolism

(below). Prostaglandins, molecules associated with localized pain, are synthesized in cells from **arachidonic acid** (see previous page) which has been cleaved from membrane lipids. The enzyme catalyzing their synthesis is known as p**rostaglandin synthase**, but is more commonly referred to as a **cyclooxygenase** (or COX) enzyme. Inhibition of the action of this enzyme is a strategy of non-steroidal pain relievers (also called **NSAID**s), such as **aspirin** or **ibuprofen**. Inhibition of the release of arachidonic acid from membranes is the mechanism of action of steroidal anti-inflammatories, which inhibit the phospholipase A$_2$ (PLA$_2$) that catalyzes the cleavage reaction.

See Kevin's YouTube lectures on Fat, Fatty Acid, and Prostaglandin Metabolism HERE, HERE, and HERE

Fatty Acid Oxidation

Breakdown of fats yields fatty acids and **glycerol**. Glycerol can be readily converted to DHAP for oxidation in glycolysis or synthesis into glucose in gluconeogenesis. Fatty acids are broken down in two carbon units of acetyl-CoA. To be oxidized, they must be transported

Prostaglandins

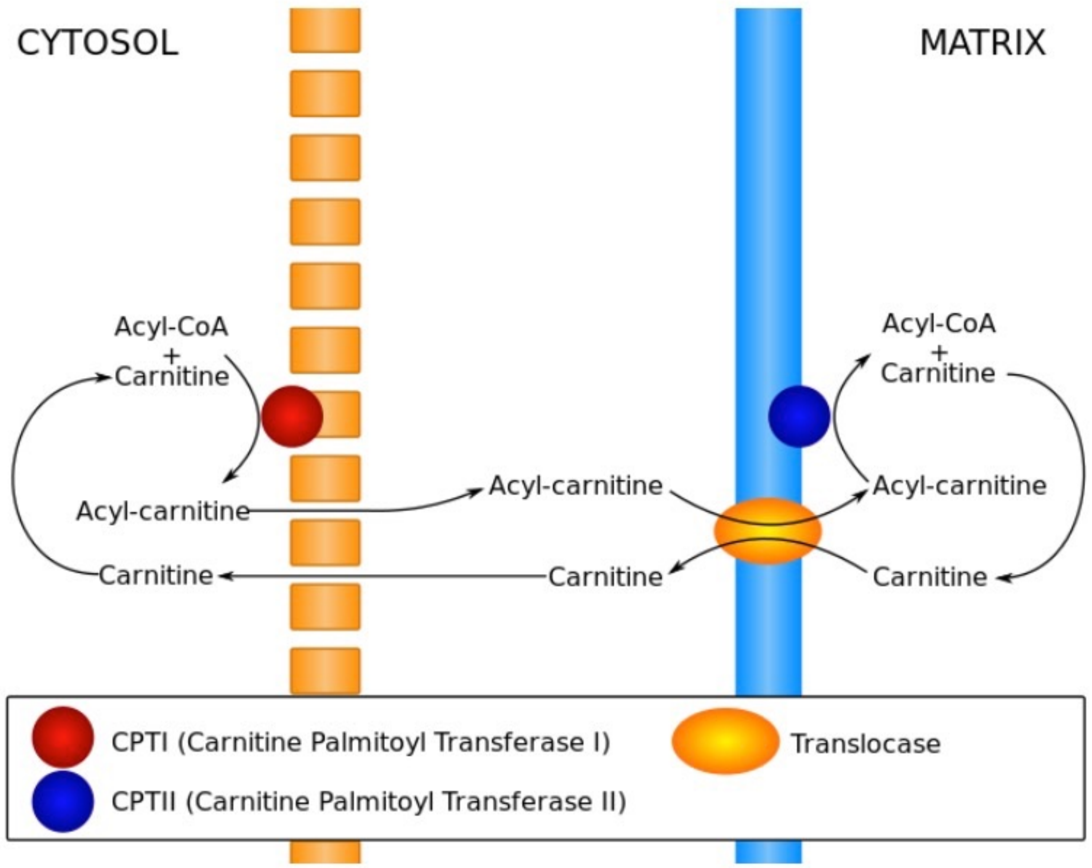
To the tune of "*Oklahoma*"

Prossss-taglandins
The ei-co-sa-noids creating pain
Are the ones to blame - when you get inflamed
And ouch(!) - they hurt inside your brain

Prossss-taglandins
Every throb and ache gets magnified
If you hope to win, cyclo-oxygen's
Generation's got to be denied
The Vioxx has all been recalled
So go get yourself Tylenol-ed

And if you aaaaaaaaaaaaache
Blame PGH synthaaaaaaaaase!
We must complain that
You make the aches prostaglandins
Prostaglandin - D2, F1, G2, E2
Prostaglandin, it's you

Recorded by Tim Karplus
Lyrics by Kevin Ahern


CYTOSOL
MATRIX
Acyl-CoA + Carnitine
Acyl-carnitine
Carnitine
Acyl-carnitine
Carnitine
Acyl-CoA + Carnitine
Acyl-carnitine
Carnitine
CPTI (Carnitine Palmitoyl Transferase I)
CPTII (Carnitine Palmitoyl Transferase II)
Translocase

Movement of Acyl-CoAs Into the Mitochondrial Matrix

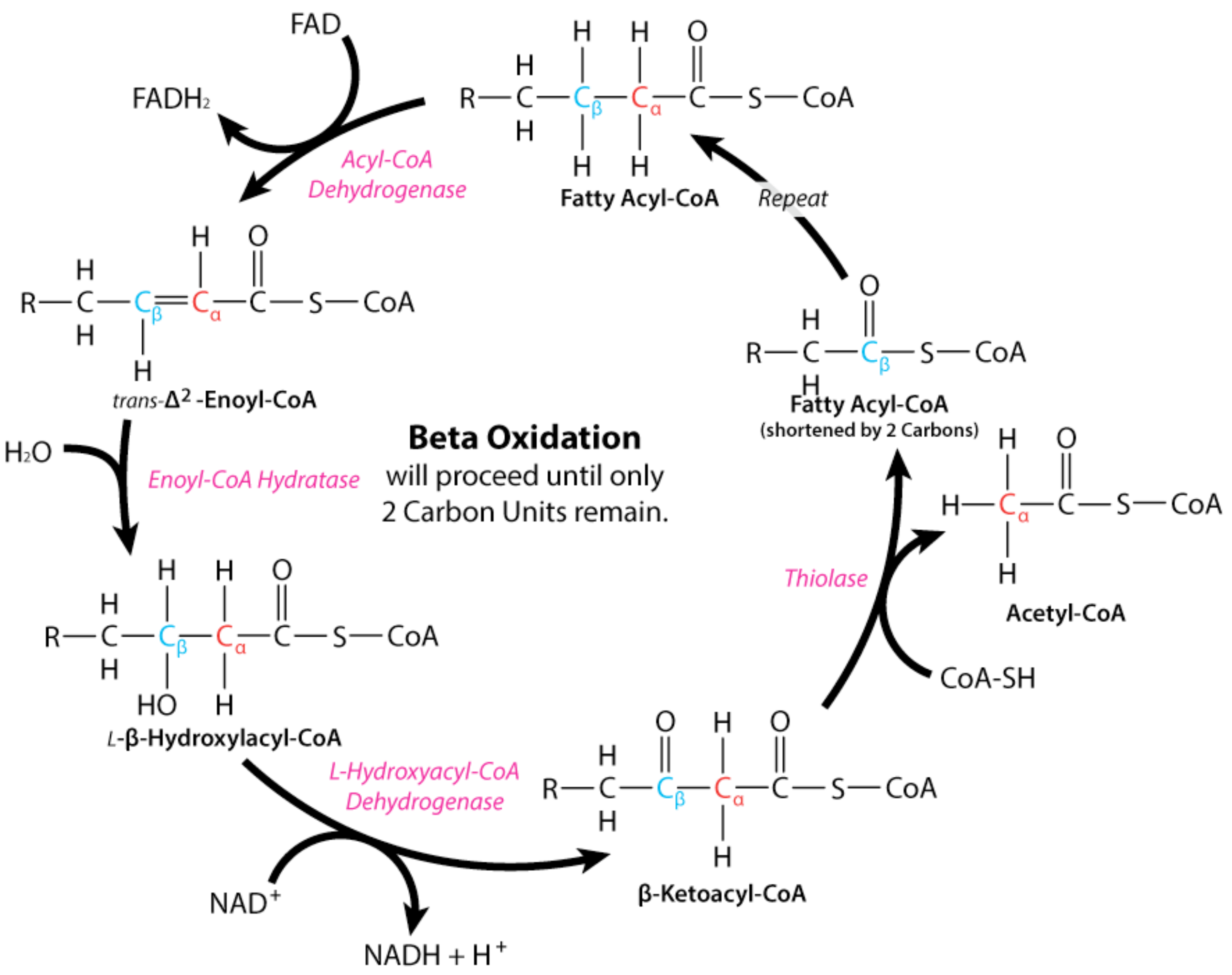
through the cytoplasm attached to coenzyme A and moved into mitochondria. The latter step requires removal of the CoA and attachment of the fatty acid to a molecule of **carnitine**. The carnitine complex is transported across the inner membrane of the mitochondrion after which the fatty acid is reattached to coenzyme A in the mitochondrial matrix.

The process of fatty acid oxidation, called **beta oxidation**, is fairly simple. The reactions all occur between carbons 2 and 3 (with #1 being the one linked to the CoA) and sequentially include the following 1) dehydrogenation to create $FADH_2$ and a fatty acyl group with a double bond in the *trans* configuration; 2) hydration across the double bond to put a hydroxyl group on carbon 3 in the L configuration; 3) oxidation of the hydroxyl group to make a ketone; and 4) thiolytic cleavage to release acetyl-CoA and a fatty acid two carbons shorter than the starting one.

Unsaturated fatty acids complicate the picture a bit (see below), primarily because they have *cis* bonds, for the most part, if they are of biological origin and these must be converted to the relevant trans intermediate made in step 1. Sometimes the bond must be moved down the chain, as well, in order to be positioned properly. Two enzymes (described below) handle all the necessary isomerizations and moves necessary to oxidize all of the unsaturated fatty acids.

Enzymes of Beta Oxidation

The reactions of fatty acid oxidation are notable in mirroring the oxidations in the latter half of the citric acid cycle – dehydrogenation of succinate to make a *trans* double bond (fumarate), hydration across the double bond to make L-malate and oxidation of the hydroxyl to make a ketone (oxaloacetate). Two of the enzymes of beta-oxidation are notable. The first is **acyl-CoA dehydrogenase**, which catalyzes the initial dehydrogenation and yields $FADH_2$. It comes in three different forms – ones that work on long, medium, or short chain length fatty acids. The first of these is sequestered in the peroxisome of


FAD
FADH₂
Acyl-CoA Dehydrogenase
Fatty Acyl-CoA
Repeat
trans-Δ² -Enoyl-CoA
Beta Oxidation
will proceed until only 2 Carbon Units remain.
Fatty Acyl-CoA (shortened by 2 Carbons)
H₂O
Enoyl-CoA Hydratase
Thiolase
Acetyl-CoA
CoA-SH
L-β-Hydroxylacyl-CoA
L-Hydroxyacyl-CoA Dehydrogenase
β-Ketoacyl-CoA
NAD⁺
NADH + H⁺

Beta Oxidation of Fatty Acids

animals whereas the others are found in the mitochondria. Plants and yeast perform beta oxidation exclusively in the peroxisome. The most interesting of the acyl-CoA dehydrogenases is the one that works on medium length fatty acids. This one, which is the one most commonly deficient in animals, has been linked to sudden infant death syndrome.

See Kevin's lectures on fat, Fatty Acid, and Prostaglandin Metabolism HERE, HERE, and HERE

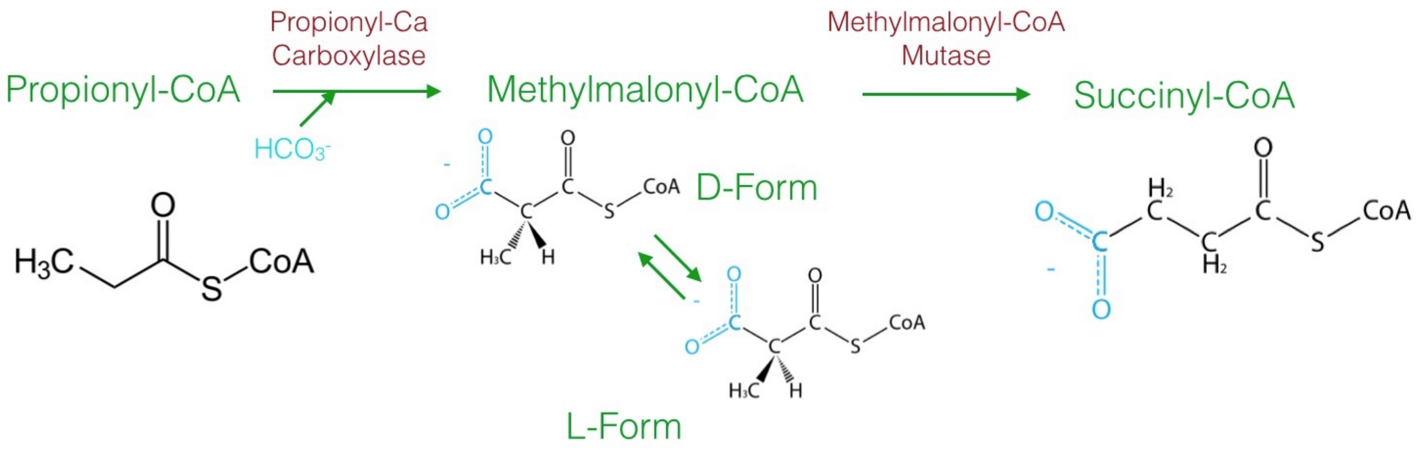
Reactions two and three in beta oxidation are catalyzed by enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase, respectively. The latter reaction yields an NADH. The final enzyme of beta oxidation is thiolase and this enzyme is notable in not only catalyzing the formation of acetyl-CoAs in beta oxidation, but also catalyzing the joining of two acetyl-CoAs (essentially the reversal of the last step of beta oxidation) to form **acetoacetyl-CoA** – essential for the pathways of ketone body synthesis and cholesterol biosynthesis.

Oxidation of Odd Chain Fatty Acids

Though most fatty acids of biological origin have even numbers of carbons, not all of them do. Oxidation of fatty acids with odd numbers of carbons ultimately produces an

In beta oxidation, it just occurred to me
The process all takes place 'tween carbons two and three
Some hydrogens are first removed to $FADH_2$
Then water adds across the bond, the H to carbon two
Hydroxyl oxidation's next, a ketone carbon three
Then thiolase catalysis dissects the last two C's
The products of the path, of course, are acetyl-CoAs
Unless there were odd carbons, hence propionyl-CoA

intermediate with three carbons called **propionyl-CoA**, which cannot be oxidized further in the beta-oxidation pathway. Metabolism of this intermediate is odd. Sequentially, the following steps occur – 1) carboxylation to make D-methylmalonyl-CoA; 2) isomerization to L-methylmalonyl-CoA; 3) rearrangement to form succinyl-CoA. The last step of the process utilizes the enzyme methylmalonyl-CoA mutase, which uses the B_{12} coenzyme in its catalytic cycle. Succinyl-CoA can then be metabolized in the citric acid cycle.

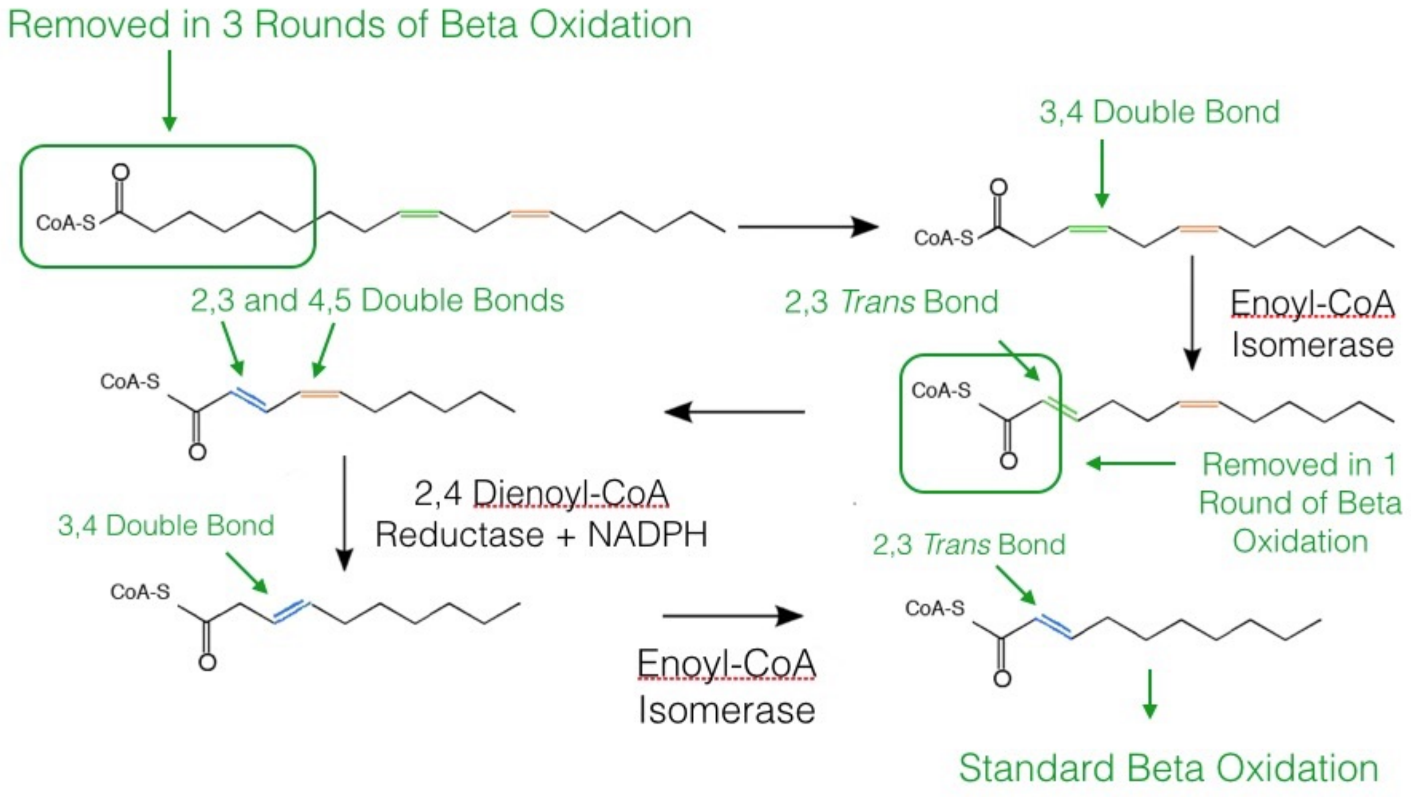


Propionyl-Ca
Carboxylase
Methylmalonyl-CoA
Mutase
Propionyl-CoA
Methylmalonyl-CoA
Succinyl-CoA
HCO_3^-
D-Form
L-Form

Odd-Chain Fatty Acid Oxidation

Unsaturated Fatty Acid Oxidation

As noted above, oxidation of unsaturated fatty acids requires two additional enzymes to the complement of enzymes for beta oxidation. If the beta oxidation of the fatty acid produces an intermediate with a *cis* bond between carbons three and four, cis-Δ3-Enoyl-CoA Isomerase will convert the bond to a *trans* bond between carbons two and three and beta oxidation can



Removed in 3 Rounds of Beta Oxidation
3,4 Double Bond
Enoyl-CoA
Isomerase
2,3 and 4,5 Double Bonds
2,3 Trans Bond
Removed in 1
Round of Beta
Oxidation
2,4 Dienoyl-CoA
Reductase + NADPH
3,4 Double Bond
2,3 Trans Bond
Enoyl-CoA
Isomerase
Standard Beta Oxidation
CoA-S

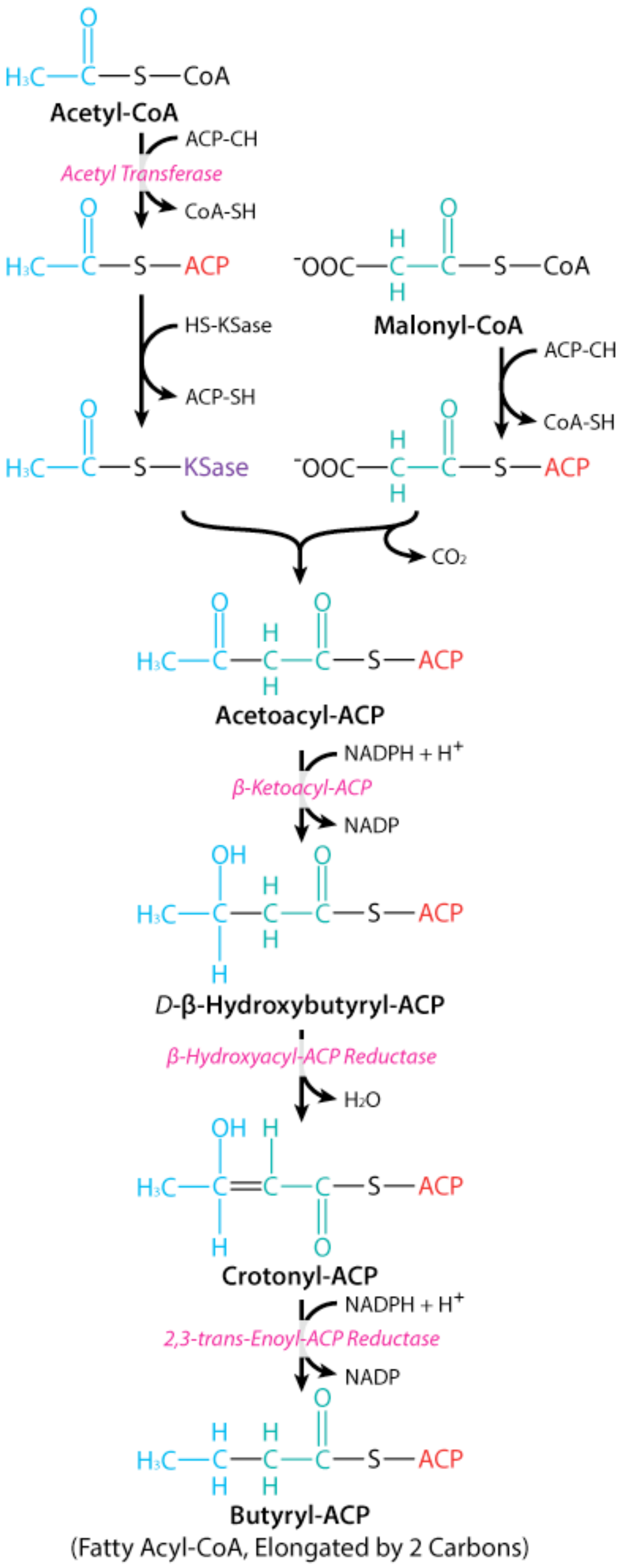
Unsaturated Fatty Acid Oxidation

proceed as normal.

On the other hand, if beta oxidation produces an intermediate with a *cis* double bond between carbons four and five, the first step of beta oxidation (dehydrogenation between carbons two and three) occurs to produce an intermediate with a *trans* double bond between carbons two and three and a *cis* double bond between carbons four and five. The enzyme 2,4 dienoyl CoA reductase reduces this intermediate (using NADPH) to one with a single *cis* bond between carbons three and four. This intermediate is then identical to the one acted on by cis-Δ3-Enoyl-CoA Isomerase above, which converts it into a regular beta oxidation intermediate, as noted above.

Alpha Oxidation

Yet another consideration for oxidation of fatty acids is alpha oxidation. This pathway is necessary for catabolism of fatty acids that have branches in their chains. For example, breakdown of chlorophyll's phytol group yields phytanic acid, which undergoes hydroxylation and oxidation on carbon number two (in contrast to carbon three of beta oxidation), followed by decarboxylation and production of a branched intermediate that can be further oxidized by the beta oxidation pathway. Though alpha oxidation is a relatively minor metabolic pathway, the inability to perform the reactions of the pathway leads to Refsum's disease where accumulation of phytanic acid leads to neurological damage.


Acetyl-CoA
ACP-CH
Acetyl Transferase
CoA-SH
HS-KSase
ACP-SH
Malonyl-CoA
ACP-CH
CoA-SH
CO₂
Acetoacyl-ACP
NADPH + H⁺
β-Ketoacyl-ACP
NADP
D-β-Hydroxybutyryl-ACP
β-Hydroxyacyl-ACP Reductase
H₂O
Crotonyl-ACP
NADPH + H⁺
2,3-trans-Enoyl-ACP Reductase
NADP
Butyryl-ACP
(Fatty Acyl-CoA, Elongated by 2 Carbons)

Fatty Acid Synthesis

Fatty Acid Synthesis

Synthesis of fatty acids occurs in the cytoplasm and endoplasmic reticulum of the cell and is chemically similar to the beta-oxidation process, but with a couple of key differences. The first of these occur in preparing substrates for the reactions that grow the fatty acid. Transport of acetyl-CoA from the mitochondria occurs when it begins to build up. Two molecules can play roles in moving it to the cytoplasm – citrate and **acetylcarnitine**. Joining of oxaloacetate with acetyl-CoA in the mitochondrion creates citrate which moves across the membrane, followed by action of citrate lyase in the cytoplasm of the cell to release acetyl-CoA and oxaloacetate. Additionally, when free acetyl-CoA accumulates in the mitochondrion, it may combine with carnitine and be transported out to the cytoplasm.

Starting with two acetyl-CoA, one is converted to **malonyl-CoA** by carboxylation catalyzed by the enzyme **acetyl-CoA carboxylase** (ACC), the only regulatory enzyme of fatty acid synthesis (see below). Next, both molecules have their CoA portions replaced by a carrier protein known as **ACP** (**acyl-carrier protein**) to form acetyl-ACP and malonyl-ACP. Joining of a fatty acyl-ACP (in this case, acetyl-ACP) with **malonyl-ACP** splits out the carboxyl that was added and creates the intermediate at the upper right in the figure at left.

From this point forward, the chemical reactions resemble those of beta oxidation reversed. First, the ketone is reduced to a hydroxyl using NADPH. In contrast to the hydroxylated intermediate of beta oxidation, the beta intermediate here is in the D-configuration. Next, water is removed from carbons 2 and 3 of the hydroxyl intermediate to produce a *trans* doubled bonded molecule. Last, the double bond is hydrogenated to yield a saturated intermediate. The process cycles with the addition of another malonyl-ACP to the growing chain until ultimately an intermediate with 16 carbons is produced (palmitoyl-CoA). At this point, the cytoplasmic synthesis ceases.

Enzymes of Fatty Acid Synthesis

Acetyl-CoA carboxylase, which catalyzes synthesis of malonyl-CoA, is the only regulated enzyme in fatty acid synthesis. Its regulation involves both allosteric control and covalent modification. The enzyme is known to be phosphorylated by both AMP Kinase and Protein Kinase A. Dephosphorylation is stimulated by **phosphatases** activated by **insulin** binding. Dephosphorylation activates the enzyme and favors its assembly into a long polymer, while phosphorylation reverses the process.

For fatty acid synthesis, I must reverse the path
Of breaking fatty acids, though you'll wonder 'bout the math

Each cycle of addition starts with carbons one two three
Yet products of reactions number carbons evenly

The answer is that CO_2 plays peek-a-boo like games
By linking to an Ac-CoA then popping off again

Reactions are like oxidations 'cept they're backwards here
Reduction, dehydration, then two hydrogens appear

The product of the process is a 16 carbon chain
The bonds are saturated, no double ones remain

For them desaturases toil to put in links of *cis*
In animals to delta nine, but no more go past this

And last there's making longer ones eicosanoidic fun
They're made by elongases in the e. reticulum

When Acids Are Synthesized

To the tune of "*When Johnny Comes Marching Home*"

The 16 carbon fatty acid, palmitate
Gets all the carbons that it needs from acetate
Which citric acid helps release
From mitochondri - matrices
Oh a shuttle's great
When acids are synthesized

Carboxylase takes substrate and it puts within
Dioxy carbon carried on a biotin
CoA's all gain a quick release
Replaced by larger ACPs
And it all begins
When acids are synthesized

A malonate contributes to the growing chain
Two carbons seven times around again, again
For saturated acyl-ates
There's lots of N-A-DPH
That you must obtain
When acids are synthesized

Palmitic acid made this way all gets released
Desaturases act to make omega-threes
The finished products big and small
Form esters with a glycerol
So you get obese
When acids are synthesized

Recorded by Tim Karplus
Lyrics by Kevin Ahern

Citrate acts as an allosteric activator and may also favor polymerization. **Palmitoyl-CoA** allosterically inactivates it.

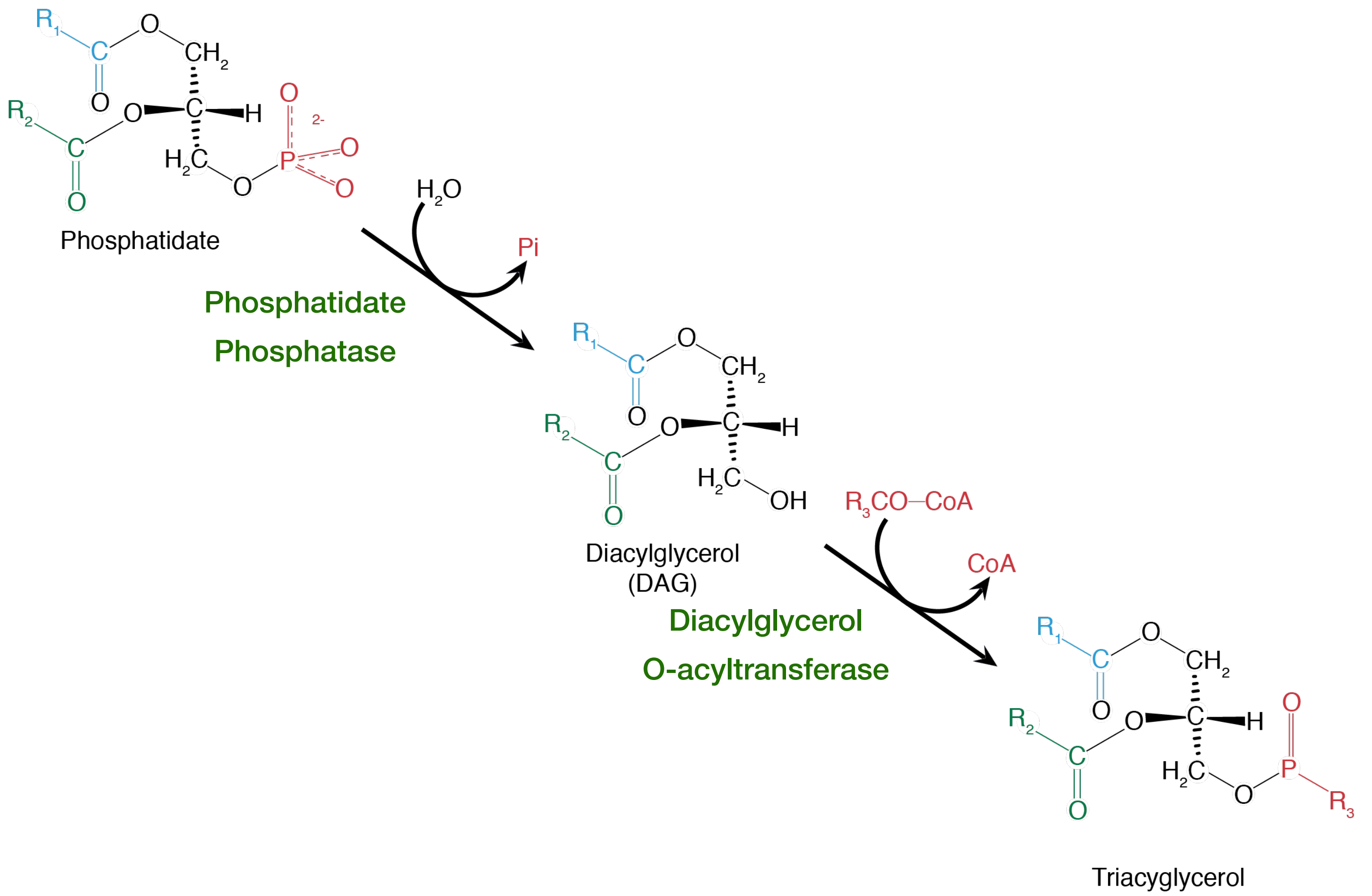
In animals, six different catalytic activities necessary for the remaining catalytic actions to fully make palmitoyl-CoA are contained in a single complex called **Fatty Acid Synthase**. These include transacylases for swapping CoA with ACP on acetyl-CoA and malonyl-CoA; a synthase to catalyze addition of the two carbon unit from the three carbon malonyl-ACP in the first step of the elongation process; a reductase to reduce the ketone; a dehydrase to catalyze removal of water, and a reductase to reduce the *trans* double bond. In bacteria, these activities are found on separate enzymes and are not part of a complex.

Fatty Acid Elongation

Elongation to make fatty acids longer than 16 carbons occurs in the endoplasmic reticulum and is catalyzed by enzymes described as **elongases**. Mitochondria also can elongate fatty acids, but their starting materials are generally shorter than 16 carbons long. The mechanisms in both environments are similar to those in the cytoplasm (a malonyl group is used to add two carbons, for example), but CoA is attached to the intermediates, not ACP. Further, whereas cytoplasmic synthesis employs an enzyme complex called fatty acid synthase, the enzymes in these organelles are separable and not part of a complex.

Desaturation of Fatty Acids

Fatty acids are synthesized in the saturated form and desaturation occurs later. Enzymes called **desaturases** catalyze the formation of *cis* double bonds in mature fatty acids. These enzymes are found in the endoplasmic reticulum. Animals are limited in the desaturated fatty acids they can make, due to an inability to catalyze reactions beyond carbons 9 and 10. Thus, humans can make **oleic acid**, but cannot synthesis **linoleic acid** or **linolenic acid**. Consequently, these two must be provided in the diet and are referred to as **essential fatty acids**.

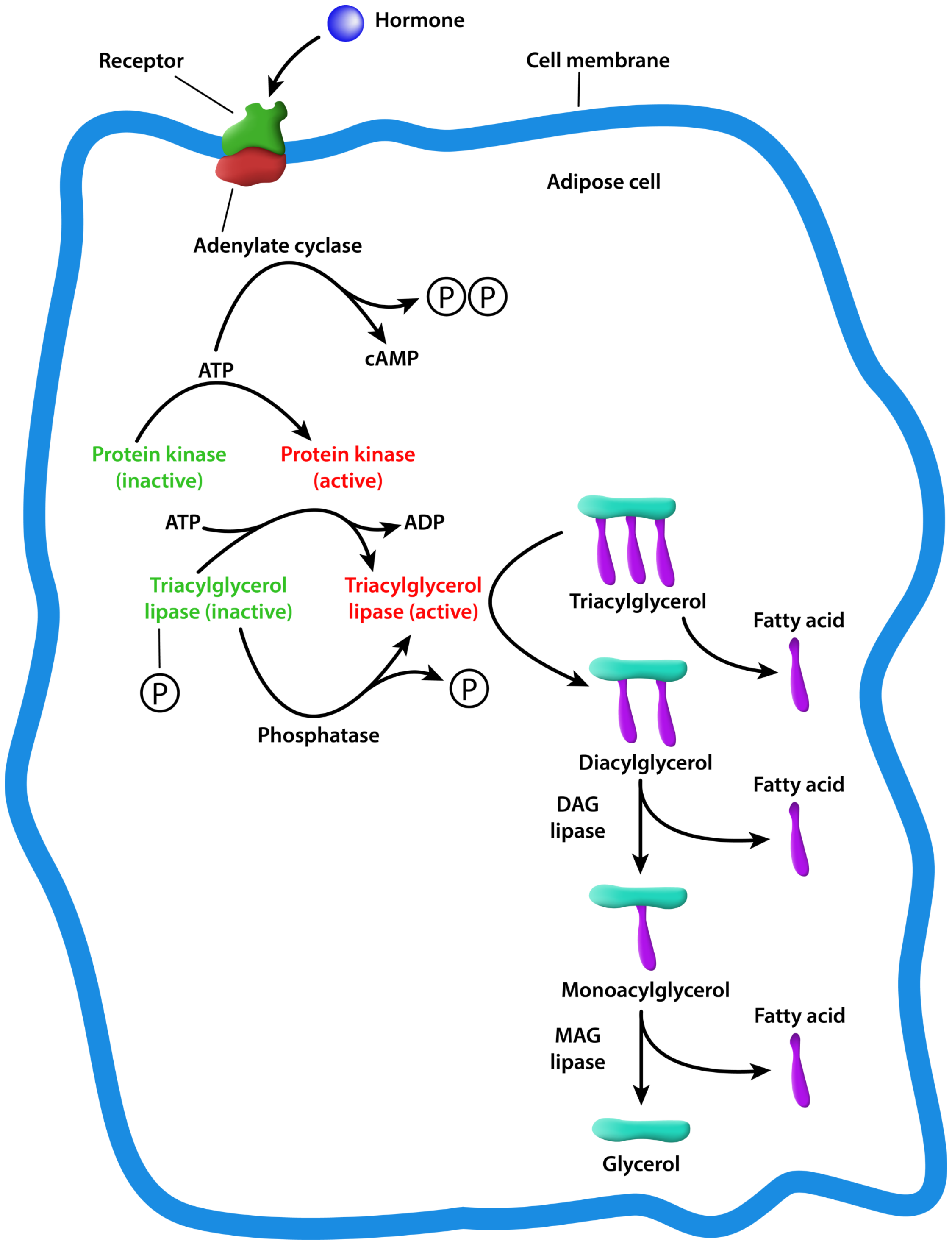


Synthesis of Fat

Metabolism of Fat

Breakdown of fat in adipocytes requires catalytic action of three enzymes, **hormone sensitive triacylglycerol lipase** (called LIPE) to remove the first fatty acid from the fat, diglyceride lipase to remove the second one and monoglyceride lipase to remove the third. Of these, only LIPE is regulated and it appears to be the rate limiting reaction. Synthesis of fat starting with glycerol-3-phosphate requires action of acyl transferase enzymes, such as glycerol-3-phosphate acyl transferase, which catalyze addition of fatty acids to the glycerol backbone.

Interestingly, there appear to be few controls of the metabolism of fatty acids. The primary control of their oxidation is availability. One way to control that is by control of the breakdown of fat. This process, which can be stimulated by the epinephrine kinase cascade, is controlled through LIPE, found in adipocytes (fat-containing cells). Breakdown of fat in apidocytes requires action of three enzymes, each


Hormone
Receptor
Cell membrane
Adipose cell
Adenylate cyclase
P P
ATP
cAMP
Protein kinase (inactive)
Protein kinase (active)
ATP
ADP
Triacylglycerol lipase (inactive)
Triacylglycerol lipase (active)
P
Phosphatase
P
Triacylglycerol
Fatty acid
Diacylglycerol
DAG lipase
Fatty acid
Monoacylglycerol
MAG lipase
Fatty acid
Glycerol

Activation of Fat Hydrolysis

hydrolyzing one fatty acid from the glycerol backbone. As noted earlier, only HSTL, which catalyzes the first hydrolysis, is regulated.

Synthesis of fat requires **glycerol-3-phosphate** (or DHAP) and three fatty acids. In the first reaction, glycerol-3-phosphate is esterified at position 1 with a fatty acid, followed by a duplicate reaction at position 2 to make **phosphatidic acid**. This molecule, which is an intermediate in the synthesis of both fats and phosphoglycerides, gets dephosphorylated to form **diacylglycerol** before the third esterification to make a fat.

Glycerophospholipid Metabolism

Phosphatidic acid, as noted above, is an important intermediate in the metabolism of glycerophospholipids. These compounds, which are important membrane constituents, can be synthesized in several ways.

Connections to Other Pathways

There are several connections between metabolism of fats and fatty acids to other metabolic pathways. As noted, phosphatidic acid is an intermediate in the synthesis of triacylglycerols, as well as of other lipids, including phosphoglycerides. Diacylglycerol (DAG), which is an intermediate in fat synthesis, also acts as a messenger in some signaling systems. Fatty acids twenty carbons long based on arachidonic acid (also called eicosanoids) are precursors of the classes of molecules known as leukotrienes and prostaglandins. The latter, in turn, are precursors of the class of molecules known as thromboxanes. The ultimate products of beta oxidation are acetyl-CoA molecules and these can be assembled by the enzyme thiolase to make acetoacetyl-CoA, which is a precursor of both ketone bodies and the isoprenoids, a broad category of compounds that include steroid hormones, cholesterol, bile acids, and the fat soluble vitamins, among others.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Chapter 7

Metabolism II

In this second section of metabolism, we cover metabolic pathways that do not have a strong emphasis on oxidation/reduction.

Metabolism II

Carbohydrate Storage and Breakdown
  - **Glycogen Breakdown**
    - **Regulation of Glycogen Metabolism**
    - **GPa/GPb Allosteric Regulation**
    - **GPa/GPb Covalent Conversion**
    - **Turning Off Glycogen Breakdown**
  - **Glycogen Synthesis**
    - **Regulation of Glycogen Synthesis**
    - **Maintaining Blood Glucose Levels**

Pentose Phosphate Pathway
Calvin Cycle
C_4 Plants
Urea Cycle
Nitrogen Fixation
Amino Acid Metabolism
Amino Acid Catabolism
Nucleotide Metabolism
Pyrimidine *de novo* Biosynthesis
Purine *de novo* Biosynthesis
Deoxyribonucleotide *de novo* Biosynthesis

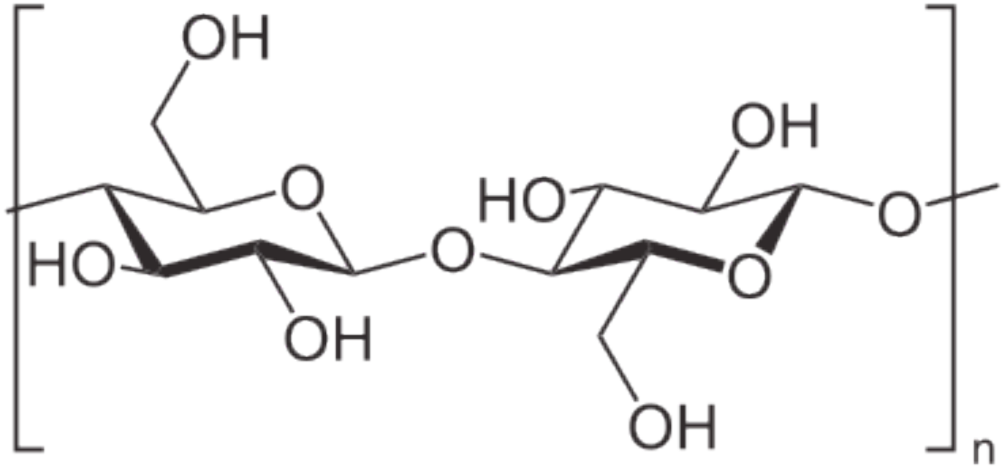
In the last chapter, we focused on metabolic pathways that played important oxidative/reductive roles relative to cellular energy. In this chapter, the pathways that we cover have lesser roles from an energy perspective, but important roles, nonetheless, in catabolism and anabolism of building blocks of proteins and nucleic acids, nitrogen balance, and sugar balance. In a sense, these might be thought of as the “kitchen sink” pathways, but it should be noted that all cellular pathways are important.

Carbohydrate Storage/Breakdown

Carbohydrates are important cellular energy sources. They provide energy quickly through glycolysis and passing of intermediates to pathways, such as the citric acid cycle, amino acid metabolism (indirectly), and the pentose phosphate pathway. It is important, therefore, to understand how these important molecules are made.

Plants are notable in storing glucose for energy in the form of amylose and amylopectin (see HERE) and for structural integrity in the form of cellulose (see HERE). These structures differ in that cellulose contains glucoses solely joined by beta-1,4 bonds, whereas amylose has only alpha1,4 bonds and amylopectin has alpha 1,4 and alpha 1,6 bonds.

Animals store glucose primary in liver and muscle in the form of a compound related to amylopectin known as glycogen. The structural differences between glycogen and amylopectin are solely due to the frequency of the alpha 1,6 branches of glucoses. In glycogen they occur about every 10 residues instead of every 30-50, as in amylopectin.



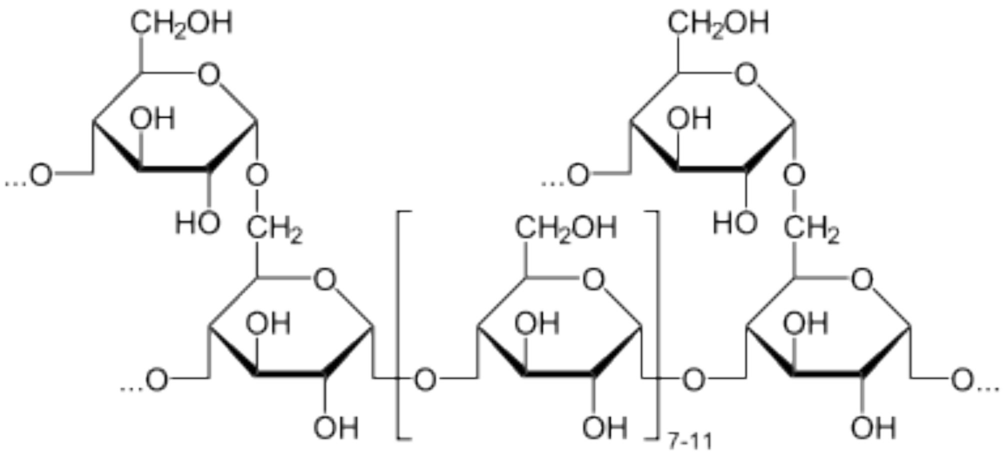
The Repeating Structure of Cellulose

Glycogen provides an additional source of glucose besides that produced *via* gluconeogenesis. Because glycogen contains so many glucoses, it acts like a battery backup for the body, providing a quick source of glucose when needed and providing a place to store excess glucose when glucose concentrations in the blood rise. The branching of glycogen is an important feature of the molecule metabolically as well. Since glycogen is broken down from the "ends" of the molecule, more branches translate to more ends, and more glucose that can be released at once.

Breakdown of glycogen involves 1) release of glucose-1-phosphate (G1P), 2) rearranging the remaining glycogen (as necessary) to permit continued breakdown, and 3) conversion of G1P to G6P for further metabolism. G6P can be 1) broken down in glycolysis, 2) converted to glucose by gluconeogenesis, and 3) oxidized in the pentose phosphate pathway.

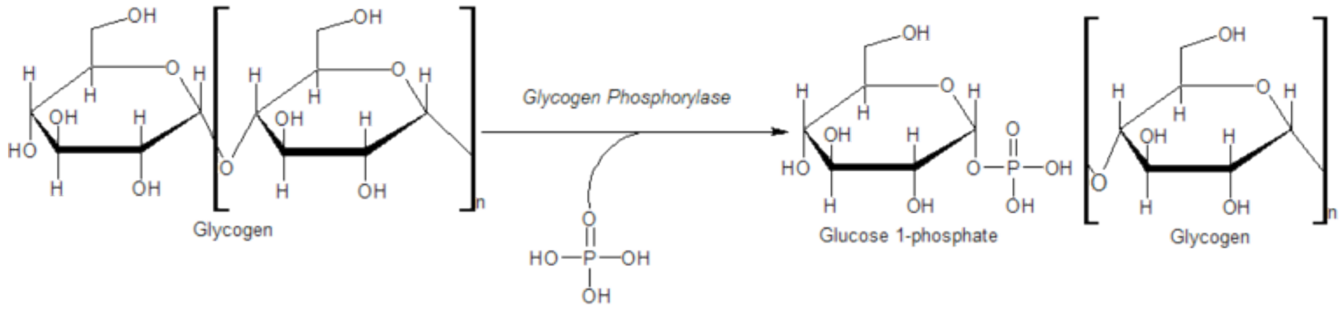
Just as in gluconeogenesis, the cell has a separate mechanism for glycogen synthesis that is distinct from glycogen breakdown. As noted previously, this allows the cell to separately control the reactions, avoiding futile cycles, and enabling a process to occur efficiently (synthesis of glycogen) that would not occur if it were simply the reversal of glycogen breakdown.

Synthesis of glycogen starts with G1P, which is converted to an 'activated' intermediate, UDP-glucose. This activated intermediate is what 'adds' the glucose to the growing glycogen



The Repeating Unit of Glycogen

chain in a reaction catalyzed by the enzyme known as glycogen synthase. Once the glucose is added to glycogen, the glycogen molecule may need to have branches inserted in it by the enzyme known as branching enzyme.



Phosphorolysis of Glycogen

Glycogen Breakdown

Glycogen phosphorylase (sometimes simply called phosphorylase) catalyzes breakdown of glycogen into Glucose-1-Phosphate (G1P). The reaction, (see above right) that produces G1P from glycogen is a phosphorolysis, not a hydrolysis reaction. The distinction is that hydrolysis reactions use water to cleave bigger molecules into smaller ones, but phosphorolysis reactions use phosphate instead for the same purpose. Note that the phosphate is just that - it does NOT come from ATP. Since ATP is *not* used to put phosphate on G1P, the reaction saves the cell energy.

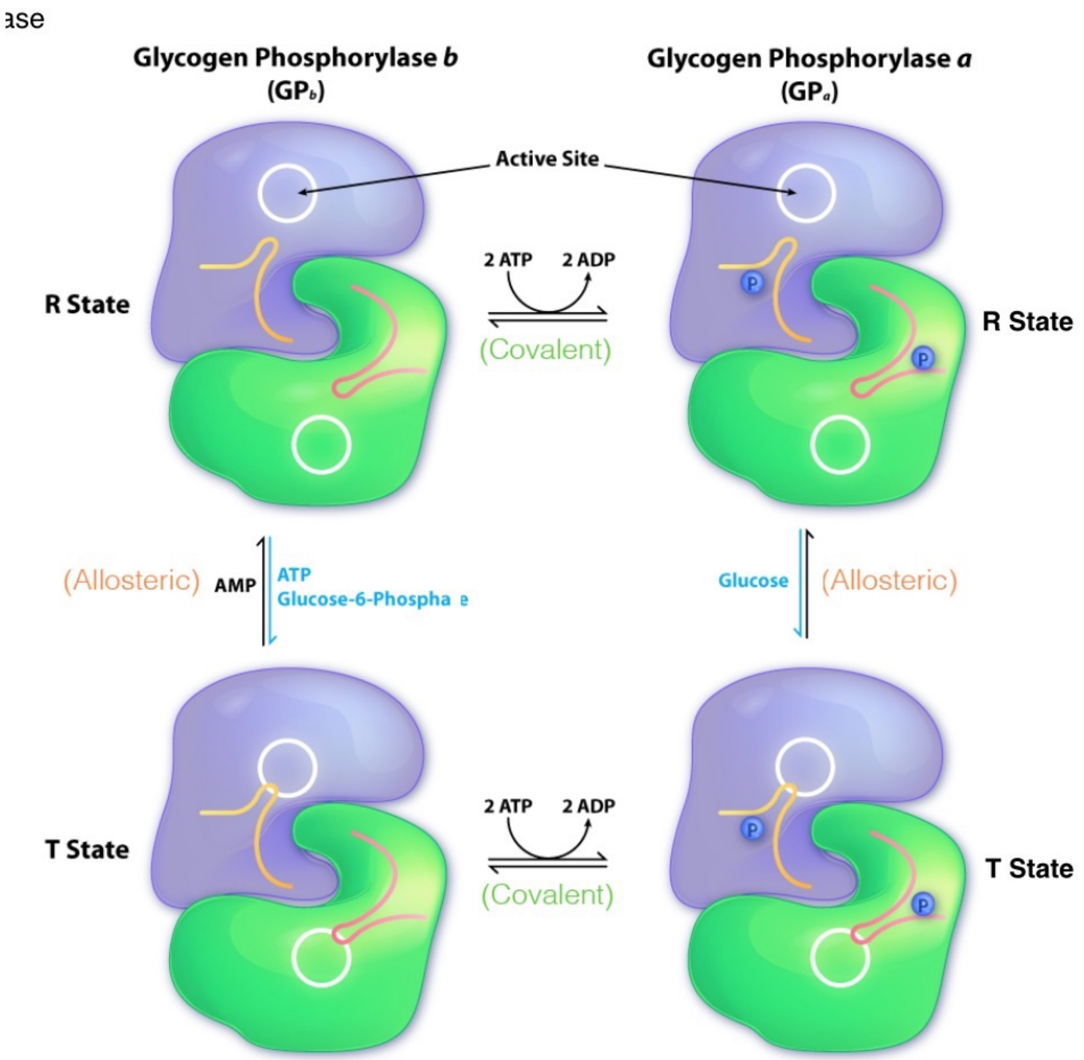
See Kevin's YouTube lectures on Glycogen Metabolism HERE, HERE, and HERE

Glycogen phosphorylase will only act on non-reducing ends of a glycogen chain that are at least 5 glucoses away from a branch point. A second enzyme, Glycogen Debranching Enzyme (GDE), is therefore needed to convert alpha(1-6) branches to alpha(1-4) branches. GDE acts on glycogen branches that have reached their limit of hydrolysis with glycogen phosphorylase. GDE acts to transfer a trisaccharide from a 1,6 branch onto an adjacent 1,4 branch, leaving a single glucose at the 1,6 branch. Note that the enzyme also catalyzes the hydrolysis of the remaining glucose at the 1,6 branch point. Thus, the breakdown products from glycogen are G1P and glucose (mostly G1P, however). Glucose can, of course, be converted to Glucose-6-Phosphate (G6P) as the first step in glycolysis by either hexokinase or glucokinase. G1P can be converted to G6P by action of an enzyme called phosphoglucomutase. This reaction is readily reversible, allowing G6P and G1P to be interconverted as the concentration of one or the other increases. This is important, because phosphoglucomutase is needed to form G1P for glycogen biosynthesis.

Regulation of Glycogen Metabolism

Regulation of glycogen metabolism is complex, occurring both allosterically and *via* hormone-receptor controlled events that result in protein phosphorylation or dephosphorylation. In order to avoid a futile cycle of glycogen synthesis and breakdown simultaneously, cells have evolved an elaborate set of controls that ensure only one pathway is primarily active at a time.

Regulation of glycogen metabolism is managed by the enzymes glycogen phosphorylase and glycogen synthase. Glycogen phosphorylase is regulated by both allosteric factors (ATP, G6P, AMP, and glucose) and by covalent modification (phosphorylation/dephosphorylation). Its regulation is consistent with the energy needs of the cell. High energy substrates (ATP, G6P, glucose) allosterically inhibit GP, while low energy substrates (AMP, others) allosterically activate it.


ase
Glycogen Phosphorylase *b*
(GP$_b$)
Glycogen Phosphorylase *a*
(GP$_a$)
Active Site
R State
2 ATP
2 ADP
(Covalent)
P
R State
(Allosteric)
AMP
ATP
Glucose-6-Phospha e
Glucose
(Allosteric)
T State
2 ATP
2 ADP
(Covalent)
P
T State

Regulation of Glycogen Phosphorylase

GPa/GPb Allosteric Regulation

Glycogen phosphorylase exists in two different covalent forms – one form with phosphate (called GPa here) and one form lacking phosphate (GPb here). GPb is converted to GPa by phosphorylation by an enzyme known as phosphorylase kinase.

GPa and GPb can each exist in an 'R' state and a 'T' state. For both GPa and GPb, the R state is the more active form of the enzyme. GPa's negative allosteric effector (glucose) is usually not abundant in cells, so GPa does not flip into the T state often. There is no positive allosteric effector of GPa, so when glucose is absent, GPa automatically flips into the R (more active) state.

GPb can convert from the T state to the GPb R state by binding AMP. Unless a cell is low in energy, AMP concentration is low. Thus GPb is not converted to the R state very often. On the other hand, ATP and/or G6P are usually present at high enough concentration in cells that GPb is readily flipped into the T state.

GPa/GPb Covalent Regulation

Because the relative amounts of GPa and GPb largely govern the overall process of glycogen breakdown, it is important to understand the controls on the enzymes that interconvert GPa and GPb. This is accomplished by the enzyme Phosphorylase Kinase, which transfers phosphates from 2 ATPs to GPb to form GPa. Phosphorylase kinase has two covalent forms – phosphorylated (active) and dephosphorylated (inactive). It is phosphorylated by the enzyme Protein Kinase A (PKA). Another way to activate the enzyme is with calcium. Phosphorylase kinase is dephosphorylated by the same enzyme, phosphoprotein phosphatase, that removes phosphate from GPa.

PKA is activated by cAMP, which is, in turn produced by adenylate cyclase after activation by a G-protein. G-proteins are activated ultimately by binding of ligands to specific 7-TM receptors, also known as G-protein coupled receptors. These are discussed in greater detail in Chapter 8. Common ligands for these receptors include epinephrine (binds beta-adrenergic receptor) and glucagon (binds glucagon receptor). Epinephrine exerts it greatest effects on muscle and glucagon works preferentially on the liver.

Turning Off Glycogen Breakdown

Turning OFF signals is as important, if not more so, than turning them ON. The steps in the glycogen breakdown regulatory pathway can be reversed at several levels. First, the ligand can leave the receptor. Second, the G-proteins have an inherent GTPase activity that serves to turn them off over time. Third, cells have phosphodiesterase (inhibited by caffeine) for breaking down cAMP. Fourth, an enzyme known as phosphoprotein phosphatase can remove phosphates from phosphorylase kinase (inactivating it) AND from GPa, converting it to the much less active GPb.

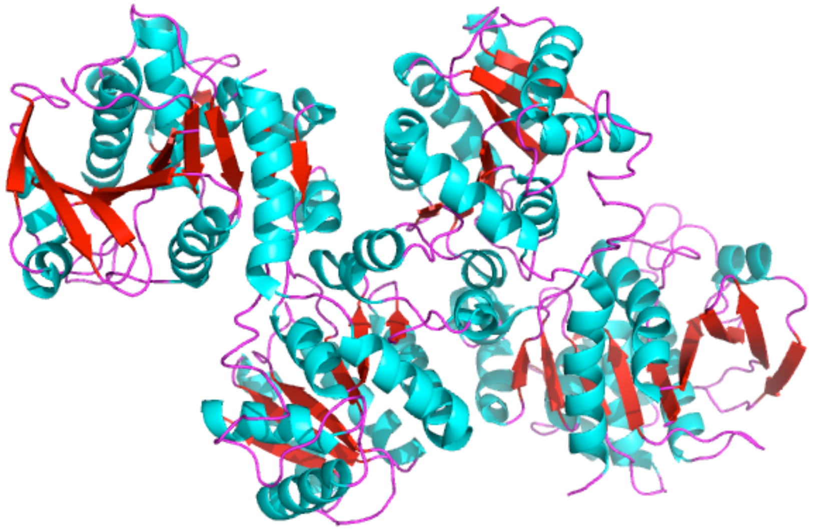
Glycogen Synthesis

The anabolic pathway contrasting with glycogen breakdown is that of glycogen synthesis. Just as cells reciprocally regulate glycolysis and gluconeogenesis to prevent a futile cycle, so too do cells use reciprocal schemes to regulate glycogen breakdown and synthesis. Let us first consider the steps in glycogen synthesis. 1) Glycogen synthesis from glucose involves phosphorylation to form G6P, and isomerization to form G1P (using phosphoglucomutase common to glycogen breakdown). G1P is reacted with UTP to form UDP-glucose in a reaction catalyzed by UDP-glucose pyrophosphorylase. Glycogen synthase catalyzes synthesis of glycogen by joining carbon #1 of the UDPG-derived glucose onto the carbon #4 of the non-reducing end of a glycogen chain. to form the familiar alpha(1,4) glycogen links. Another product of the reaction is UDP.

It is also worth noting in passing that glycogen synthase will only add glucose units from UDPG onto a preexisting glycogen chain that has at least four glucose residues. Linkage of the first few glucose units to form the minimal "primer" needed for glycogen synthase recognition is catalyzed by a protein called glycogenin, which attaches to the first glucose and catalyzes linkage of the first eight glucoses by alpha(1,4) bonds. 3) The characteristic alpha(1,6) branches of glycogen are the products of an enzyme known as Branching Enzyme. Branching Enzyme breaks alpha(1,4) chains and carries the broken chain to the carbon #6 and forms an alpha(1,6) linkage.

Regulation of Glycogen Synthesis

The regulation of glycogen biosynthesis is reciprocal to that of glycogen breakdown. It also has a cascading covalent modification system similar to the glycogen breakdown system described above. In fact, part of the system is identical to glycogen breakdown. Epinephrine or glucagon signaling can stimulate adenylate cyclase to make cAMP, which activates PKA, which activates phosphorylase kinase.



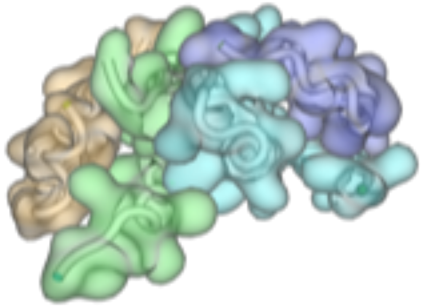
Glycogen Synthase

In glycogen breakdown, phosphorylase kinase phosphorylates GPb to the more active form, GPa. In glycogen synthesis, protein kinase A phosphorylates the active form of glycogen synthase (GSa), and converts it into the usually inactive b form (called GSb). Note the conventions for glycogen synthase and glycogen phosphorylase. For both enzymes, the more active forms are called the 'a' forms (GPa and GSa) and the less active forms are called the 'b' forms (GPb and GSb). The *major* difference, however, is that GPa has a phosphate, but GSa does not and GPb has no phosphate, but GSb does. Thus phosphorylation and dephosphorylation have *opposite effects* on the enzymes of glycogen metabolism. This is the hallmark of reciprocal regulation. It is of note that the less active glycogen synthase form, GSb, can be activated by G6P. Recall that G6P had the exactly opposite effect on GPb.

See Kevin's YouTube lectures on Glycogen Metabolism HERE, HERE, and HERE

Glycogen synthase, glycogen phosphorylase (and phosphorylase kinase) can be dephosphorylated by several enzymes called phosphatases. One of these is called Protein Phosphatase and it is activated when insulin binds to a receptor in the cell membrane. It causes PP to be activated, stimulating dephosphorylation,

Interactive 7.1



The 3D Structure of Insulin

and thus activating glycogen synthesis and inhibiting glycogen breakdown. Again, there is reciprocal regulation of glycogen synthesis and degradation.

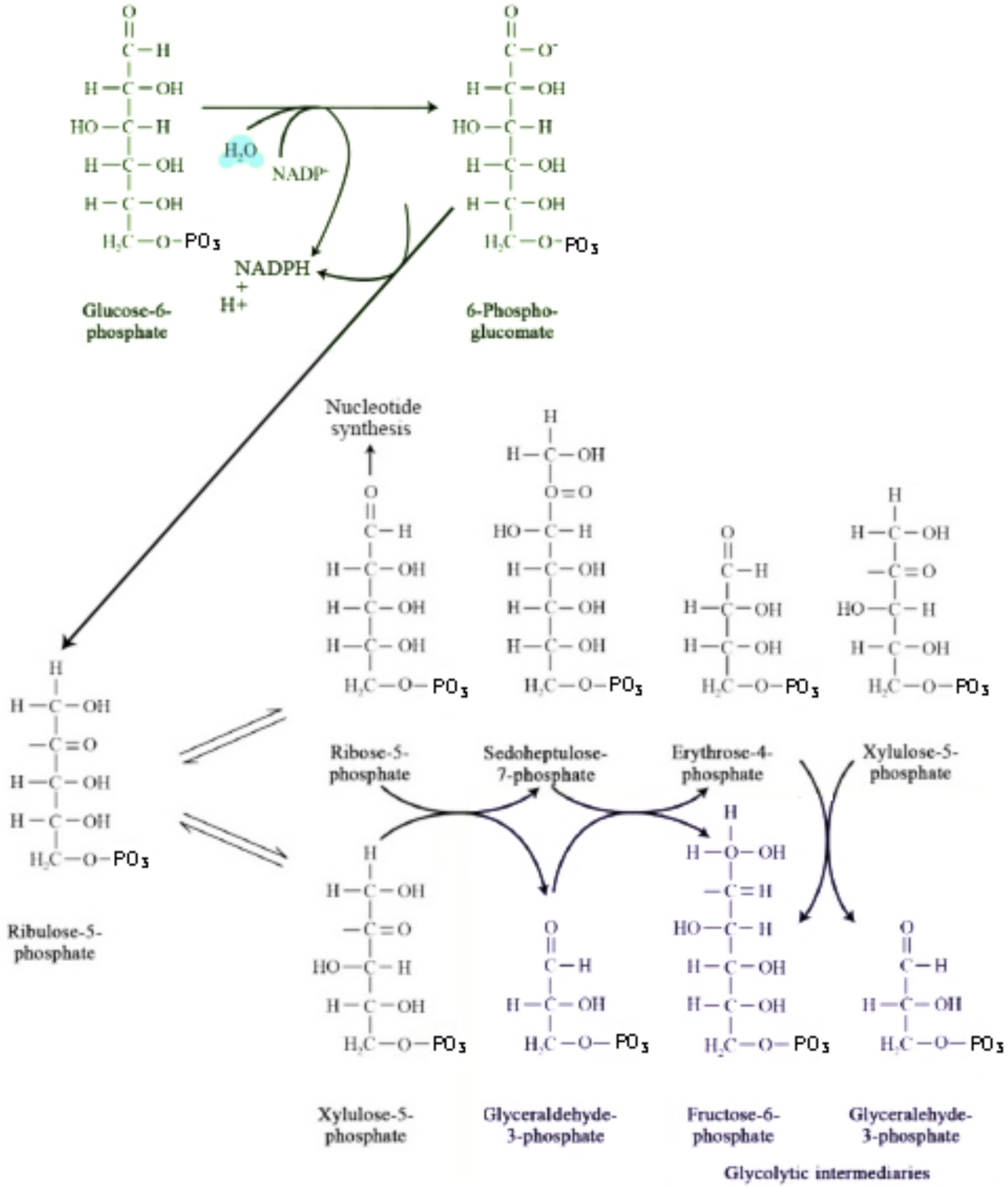
Maintaining Blood Glucose Levels

After a meal, blood glucose levels rise and insulin is released. It simultaneously stimulates uptake of glucose by cells and incorporation of it into glycogen by activation of glycogen synthase and inactivation of glycogen phosphorylase. When blood glucose levels fall, GPa gets activated (stimulating glycogen breakdown to raise blood glucose) and GSb is formed (stopping glycogen synthesis).

Pentose Phosphate Pathway

The Pentose Phosphate Pathway (PPP) is one that many students are confused by. Perhaps the reason for this is that it doesn’t really have a single direction in which it proceeds, as will be apparent below.

Portions of the PPP are similar to the Calvin Cycle of plants, also known as the dark reactions of photosynthesis. We discuss these reactions separately in the next section. The primary functions of the PPP are to produce NADPH (for use in anabolic reductions), **ribose-5-phosphate** (for making nucleotides), and **erythrose-4-phosphate** (for making aromatic amino


Glucose-6-phosphate
H_2O
NADP+
NADPH + H+
6-Phospho-glucomate
Nucleotide synthesis
Ribulose-5-phosphate
Ribose-5-phosphate
Sedoheptulose-7-phosphate
Erythrose-4-phosphate
Xylulose-5-phosphate
Xylulose-5-phosphate
Glyceraldehyde-3-phosphate
Fructose-6-phosphate
Glyceralehyde-3-phosphate
Glycolytic intermediaries

Pentose Phosphate Pathway

acids). Three molecular intermediates of glycolysis can funnel into PPP (or be used as usual in glycolysis). They include G6P, fructose-6-phosphate (in two places), and glyceraldehyde-3-phosphate (also in two places).

A starting point for the pathway (though there are other entry points) is the oxidative phase. It includes two reactions generating NADPH. In the first of these, oxidation of glucose-6-phosphate (catalyzed by **glucose-6-phosphate dehydrogenase**), produces NADPH and 6-phosphogluconolactone. 6-phosphogluconolactone spontaneously gains water and loses a proton to become **6-phosphogluconate**. Oxidation of this produces **ribulose-5-phosphate** and another NADPH and releases CO_2. The remaining steps of the pathway are known as the non-oxidative phase and involve interconversion of sugar phosphates.

I need erythrose phosphate
And don’t know what to do
My cells are full of G-6-P
Plus NADP too

But I just hit upon a plan
As simple as can be
I’ll run reactions through the path
That’s known as PPP

In just two oxidations
There’s ribulose-5P
Which morphs to other pentoses
Each one attached to P

The next step it is simple
Deserving of some praise
The pentose carbons mix and match
Thanks to transketolase

Glyceraldehyde’s a product
Sedoheptulose is too
Each with a trailing phosphate
But we are not quite through

Now three plus seven is the same
As adding six and four
By swapping carbons back and forth
There’s erythrose-P and more

At last I’ve got the thing I need
From carbons trading places
I’m happy that my cells are full
Of these transaldolases

For example, ribulose-5-phosphate is converted to ribose-5-phosphate (R5P) by the enzyme **ribulose-5-phosphate isomerase**. Alternatively, ribulose-5-phosphate can be converted to **xylulose-5-phosphate** (Xu5P). R5P and Xu5P (10 carbons total) can be combined and rearranged by **transketolase** to produce intermediates with 3 and 7 carbons (glyceraldehyde-3-phosphate and **sedoheptulose-7-phosphate**, respectively). These last two molecules can, in turn be rearranged by **transaldolase** into 6 and 4 carbon sugars (fructose-6-phosphate and erythrose-4-phosphate, respectively). Further, the erythrose-4-phosphate can swap parts with Xu5P to create glyceraldehyde-3-phosphate and fructose-6-phosphate.

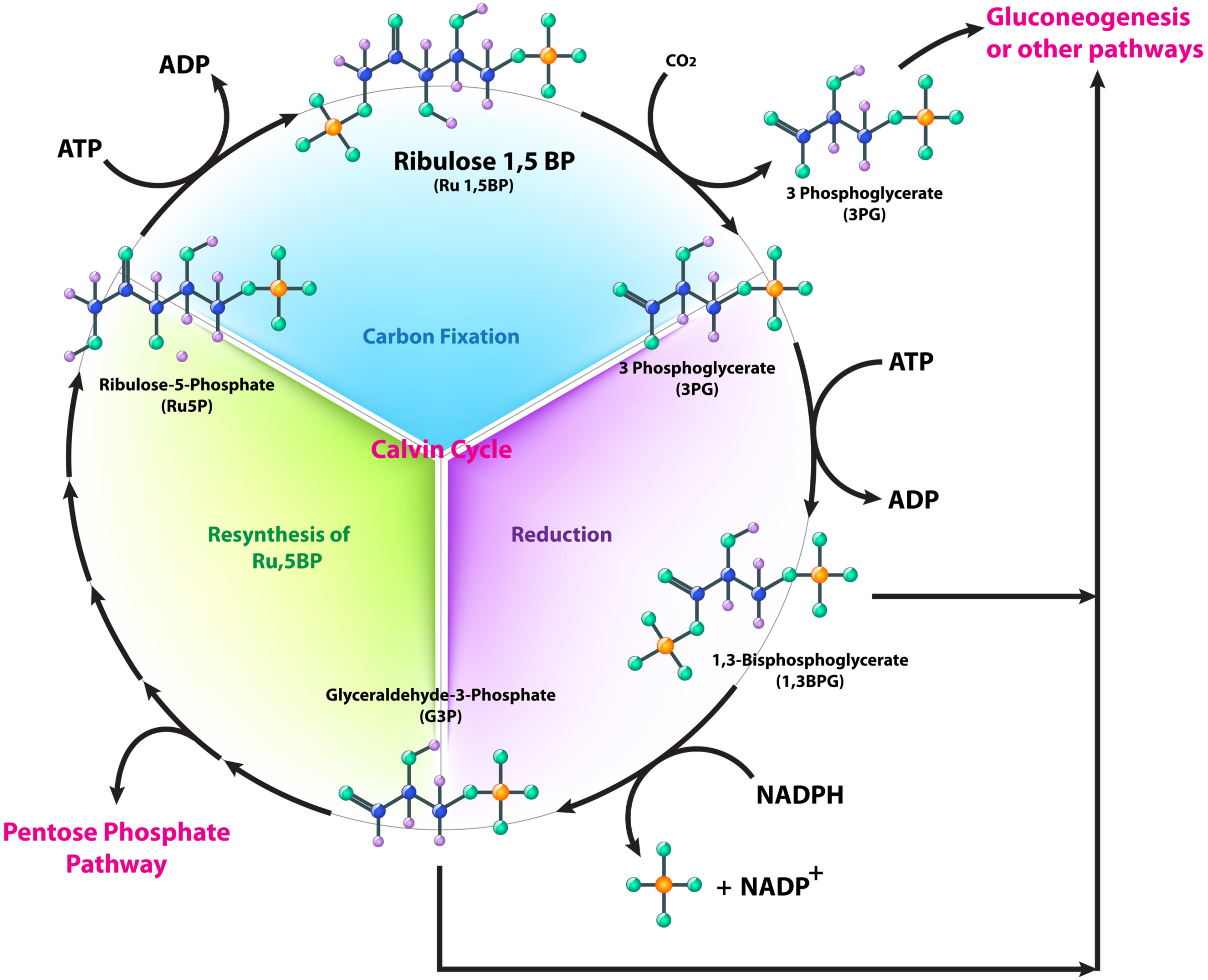
It is important to recognize that the

PPP pathway is not a “top-down” pathway, with all the intermediates derived from a starting G6P. All of the reactions are reversible, so that, for example, fructose-6-phosphate and glyceraldehyde-3-phosphate from glycolysis can reverse the last reaction of the previous paragraph to provide a means of synthesizing ribose-5-phosphate non-oxidatively. The pathway also provides a mechanism to cells for metabolizing sugars, such as Xu5P and ribulose-5-phosphate. In the bottom line of the pathway, the direction the pathway goes and the intermediates it produces are determined by the needs of, and intermediates available to, the cell.

As noted above, the pathway connects in three places with glycolysis. In non-plant cells, the PPP pathway occurs in the cytoplasm (along with glycolysis), so considerable “intermingling” of intermediates can and does occur. Erythrose-4-phosphate is an important precursor of aromatic amino acids and ribose-5-phosphate is an essential precursor for making nucleotides.

Calvin Cycle

The Calvin Cycle occurs exclusively in photosynthetic organisms and is the part of photosynthesis referred to as the “Dark Cycle.”


ADP
ATP
Ribulose 1,5 BP
(Ru 1,5BP)
CO2
Gluconeogenesis
or other pathways
3 Phosphoglycerate
(3PG)
Carbon Fixation
Ribulose-5-Phosphate
(Ru5P)
3 Phosphoglycerate
(3PG)
ATP
Calvin Cycle
ADP
Resynthesis of
Ru,5BP
Reduction
1,3-Bisphosphoglycerate
(1,3BPG)
Glyceraldehyde-3-Phosphate
(G3P)
NADPH
+ NADP+
Pentose Phosphate
Pathway

The Calvin Cycle

It is in this part of the process that carbon dioxide is taken from the atmosphere and ultimately built into glucose (or other sugars). Though reduction of carbon dioxide to glucose ultimately requires electrons from twelve molecules of NADPH (and 18 ATPs), it is a bit confusing because one reduction occurs 12 times (1,3 BPG to G3P) to achieve the reduction necessary to make one glucose.

One of the reasons students find the pathway a bit confusing is because the carbon dioxides are absorbed one at a time into six different molecules of **ribulose-1,5-bisphosphate** (Ru1,5BP). At no point are the six carbons ever together in the same molecule to make a single glucose. Instead, six molecules of Ru1,5BP (30 carbons) gain six more carbons via carbon dioxide and then split into 12 molecules of 3-phosphoglycerate (36 carbons). The gain of six carbons

Photosynthesis is Divine

To the tune of "*Scarborough Fair*"

Photosynthesis is divine
Fixing carbon using sunshine
It's thanks to plants that we've got a prayer
They pull CO_2 from the air

Reaping energy from the sun
It's efficient second to none
You grab the photons almost at will
Protoporphyrin chlorophyll

Light reactions of System II
Split up water, making O_2
Electrons pass through schemes labeled 'Z'
Pumping protons gradiently

ATP's made due to a shift
Of the protons spinning quite swift
An enzyme turbine, cellular maze
You know as A-T-P synthase

Carbon's fixed onto a substrate
Ribulose-1,5-bisphosphate
Rubisco acts in-e-fficient-ly
Splitting it into 3PGs

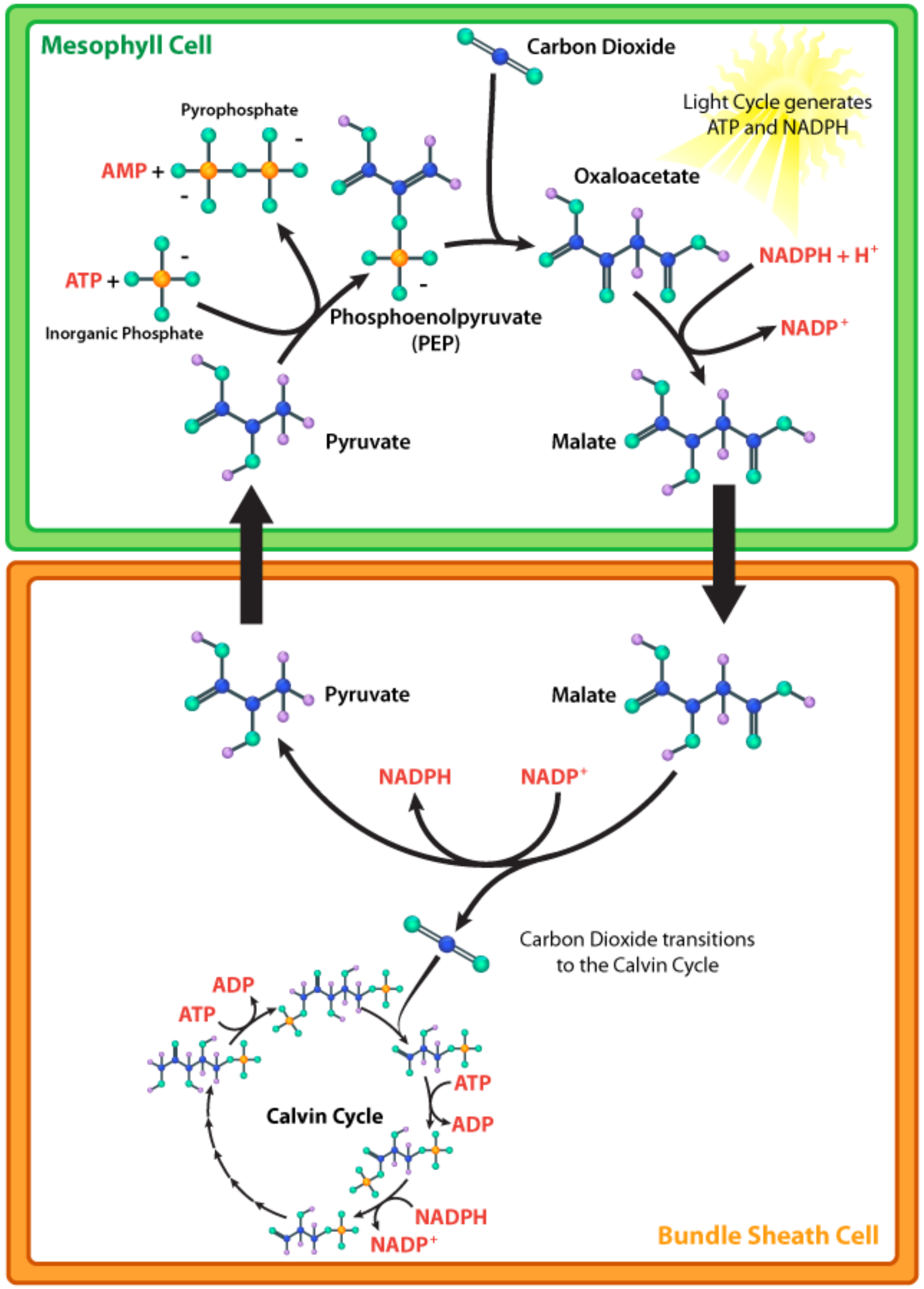
If the enzyme grabs an O_2
It makes glycolate, it is true
The Calvin Cycle works in a wheel
Giving plants a sugary meal

So photosynthesis is divine
'Cause it happens all of the time
From dawn to dusk and times in between
Solar panels truly are green

Recording by David Simmons
Lyrics by Kevin Ahern

allows two three carbon molecules to be produced in excess for each turn of the cycle. These two molecules molecules are then converted into glucose using the enzymes of gluconeogenesis.

Like the citric acid cycle, the Calvin Cycle doesn't really have a starting or ending point, but can we think of the first reaction as the fixation of carbon dioxide to Ru1,5BP. This reaction is catalyzed by the enzyme known as ribulose-1,5bisphosphate carboxylase (RUBISCO). The resulting six carbon intermediate is unstable and each Ru1,5BP is rapidly converted to 3-phosphoglycerate. As noted, if one starts with 6 molecules of Ru1,5BP and makes 12 molecules of 3-PG, the extra 6 carbons that are a part of the cycle can be shunted off as two three-carbon molecules of glyceraldehyde-3-phosphate (GA3P) to gluconeogenesis, leaving behind 10 molecules of GA3P to be reconverted into 6 molecules of Ru1,5BP. That part of the pathway requires multiple steps, but only utilizes two enzymes unique to plants - **sedoheptulose-1,7bisphosphatase** and **phosphoribulokinase**. **RUBISCO** is the third enzyme of the pathway that is unique to plants. All of the other enzymes of the pathway are common to plants and animals and include some found in the pentose phosphate pathway and gluconeogenesis.


Mesophyll Cell
Carbon Dioxide
Light Cycle generates ATP and NADPH
Pyrophosphate
AMP +
ATP +
Inorganic Phosphate
Oxaloacetate
NADPH + H⁺
NADP⁺
Phosphoenolpyruvate (PEP)
Pyruvate
Malate
Pyruvate
Malate
NADPH
NADP⁺
Carbon Dioxide transitions to the Calvin Cycle
ADP
ATP
Calvin Cycle
ATP
ADP
NADPH
NADP⁺
Bundle Sheath Cell

C4 Plant Cycle

C_4 Plants

The Calvin Cycle is the means by which plants assimilate carbon dioxide from the atmosphere, ultimately into glucose. Plants use two general strategies for doing so. The first is employed by plants called C_3 plants (most plants) and it simply involves the pathway described above. Another class of plants, called C_4 plants employ a novel strategy for concentrating the

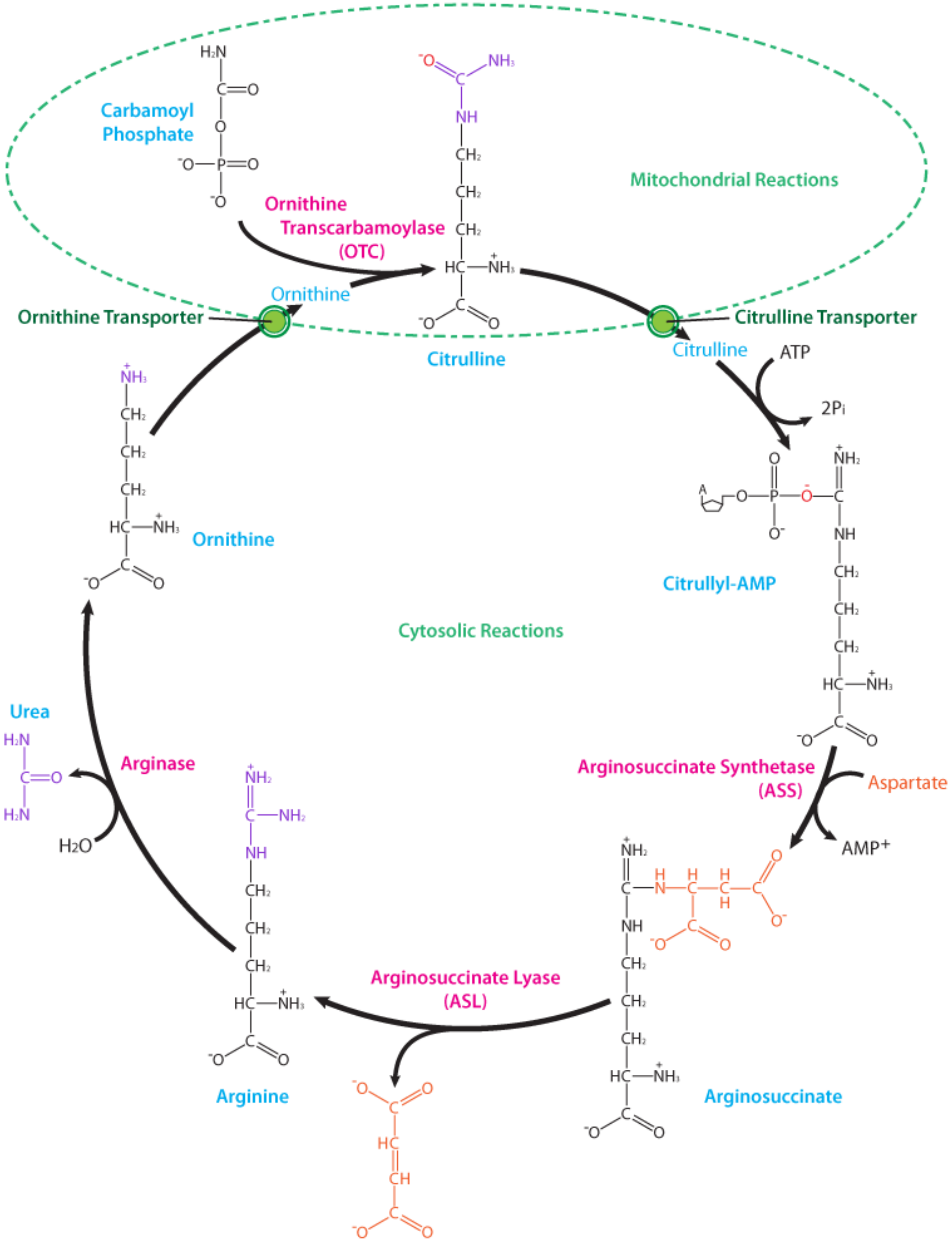
Watch Kevin's YouTube lecture on Photosynthesis HERE

CO_2 prior to assimilation. C_4 plants are generally found in hot, dry environments where conditions favor the wasteful photorespiration reactions of RUBISCO, as well as loss of water. In these plants, carbon dioxide is captured in special mesophyll cells first by phosphoenolpyruvate (PEP) to make oxaloacetate. The oxaloacetate is converted to malate and transported into bundle sheath cells where the carbon dioxide is released and it is captured by ribulose-1,5-bisphosphate, as in C_3 plants and the Calvin Cycle proceeds from there. The advantage of this scheme is that it allows concentration of carbon dioxide while minimizing loss of water and photorespiration.

Urea Cycle

Yet another cyclic pathway important in cells is the urea cycle (next page). With reactions spanning the cytoplasm and the mitochondria, the urea cycle occurs mostly in the liver and kidney. The cycle plays an important role in nitrogen balance in cells and is found in organisms that produce **urea** as a way to excrete excess amines.

The cycle scavenges free ammonia (as


Carbamoyl Phosphate
Ornithine Transcarbamoylase (OTC)
Mitochondrial Reactions
Ornithine
Ornithine Transporter
Citrulline
Citrulline Transporter
Citrulline
ATP
2Pi
Citrullyl-AMP
Ornithine
Cytosolic Reactions
Urea
Arginase
H2O
Arginosuccinate Synthetase (ASS)
Aspartate
AMP+
Arginosuccinate Lyase (ASL)
Arginine
Arginosuccinate

The Urea Cycle

They Call The Stuff Urea

To the tune of “*They Call The Wind Mariah*”

Get ATPs, bicarbonate,
Ammonia catalyzing
To make carba-mo-yl phosphate
And then start the synthesizing

When joined up with an ornithine
In THE mi-TOE-chon-DREE-a
It turns into a citrulline
When cycling to urea

Urea!
Urea!
They call the stuff urea!

On exit to the cytosol
There's bonding aspartat-ic
The argininosuccinate
Is produced in this schematic

Bid farewell to a fumarate
Amino panacea
Arises when the arginine
Gets lysed to form urea

Urea!
Urea!
You’ve just made some urea!

The body handles many things
Requiring its attention
Like balancing aminos for
Uremia prevention

So if there's excess nitrogen
It is a good idea
To rid yourself of surplus by
Producing some urea

Urea!
Urea!
Go out and take a pee, yeah!

Urea!
Urea!
Have yourself a pee, yeah!

Recorded by David Simmons
Lyrics by Kevin Ahern

ammonium ion) which is toxic if it accumulates. The capture reaction also requires ATP, and bicarbonate, and the product is **carbamoyl phosphate**. This molecule is combined with the non-protein amino acid known as **ornithine** to make another non-protein amino acid known as **citrulline**. Addition of aspartate to citrulline creates **argninosuccinate**, which splits off a fumarate, creating arginine (a source of arginine). If arginine is not needed, it can be hydrolyzed to yield urea (excreted) and ornithine, thus completing the cycle.

The first two reactions described here occur in the mitochondrion and the remaining ones occur in the cytoplasm. Molecules of the urea cycle intersecting other pathways include fumarate (citric acid cycle), aspartate (amino acid metabolism), arginine (amino acid metabolism), and ammonia (amino acid metabolism).

Nitrogen Fixation

The process of nitrogen fixation is important for life on earth, because atmospheric nitrogen is ultimately the source of amines in proteins and DNA. The enzyme playing an important role in this process is called nitrogenase and it is found in certain types of anaerobic bacteria called diazotrophs. Symbiotic relationships between some plants (legumes, for example) and the nitrogen-fixing bacteria provide the plants with access to reduced nitrogen. The overall reduction reaction catalyzed by nitrogenase is

$$N_2 + 6H^+ + 6e^- \rightarrow 2NH_3$$

In these reactions, the hydrolysis of 16 ATP is required. The ammonia can be assimilated into glutamate and other molecules. Enzymes performing nitrogenase catalysis are very susceptible to oxygen and must be kept free of it. It is for this reason that most nitrogen-fixing bacteria are anaerobic. Movement of amines through biological systems occurs largely by the process of transmination, discussed below in amino acid metabolism.

Four Families of Synthesis of Essential Amino Acids

1. Aspartate – lys, met, thr – proceed through aspartyl-beta-phosphate (catalyzed by aspartokinase)

2. Pyruvate – leu, ile, val – proceed through hydroxyethyl-TPP intermediate

3. Aromatic – phe, tyr, trp – precursors are PEP and erythrose-4-phosphate

4. Histidine – from 5-phosphoribosyl-pyrophosphate (from erythrose-4-phosphate), histidine (from ribose-5-phosphate),

Amino Acid Metabolism

The pathways for the synthesis and degradation of amino acids used in proteins are the most varied among the reactions synthesizing biological building blocks. We start with some terms. First, not all organisms can synthesize all the amino acids they need. Amino acids that an organism cannot synthesize (and therefore must have in their diets) are called **essential amino acids**. The remaining amino acids that the body can synthesize are called non-essential.

Amino acids are also divided according to the pathways involved in their degradation. There are three general categories. Ones that yield intermediates in the glycolysis pathway are called **glucogenic** and those that yield intermediates of acetyl-CoA or acetoacetate are called **ketogenic**. Those that involve both are called glucogenic and ketogenic.

See Kevin's YouTube lectures on Nitrogen Metabolism HERE and HERE

An important general consideration in amino acid metabolism is that of transamination. In this process, an exchange of amine and oxygen between an amino acid and an alpha-ketoacid occurs (see below)

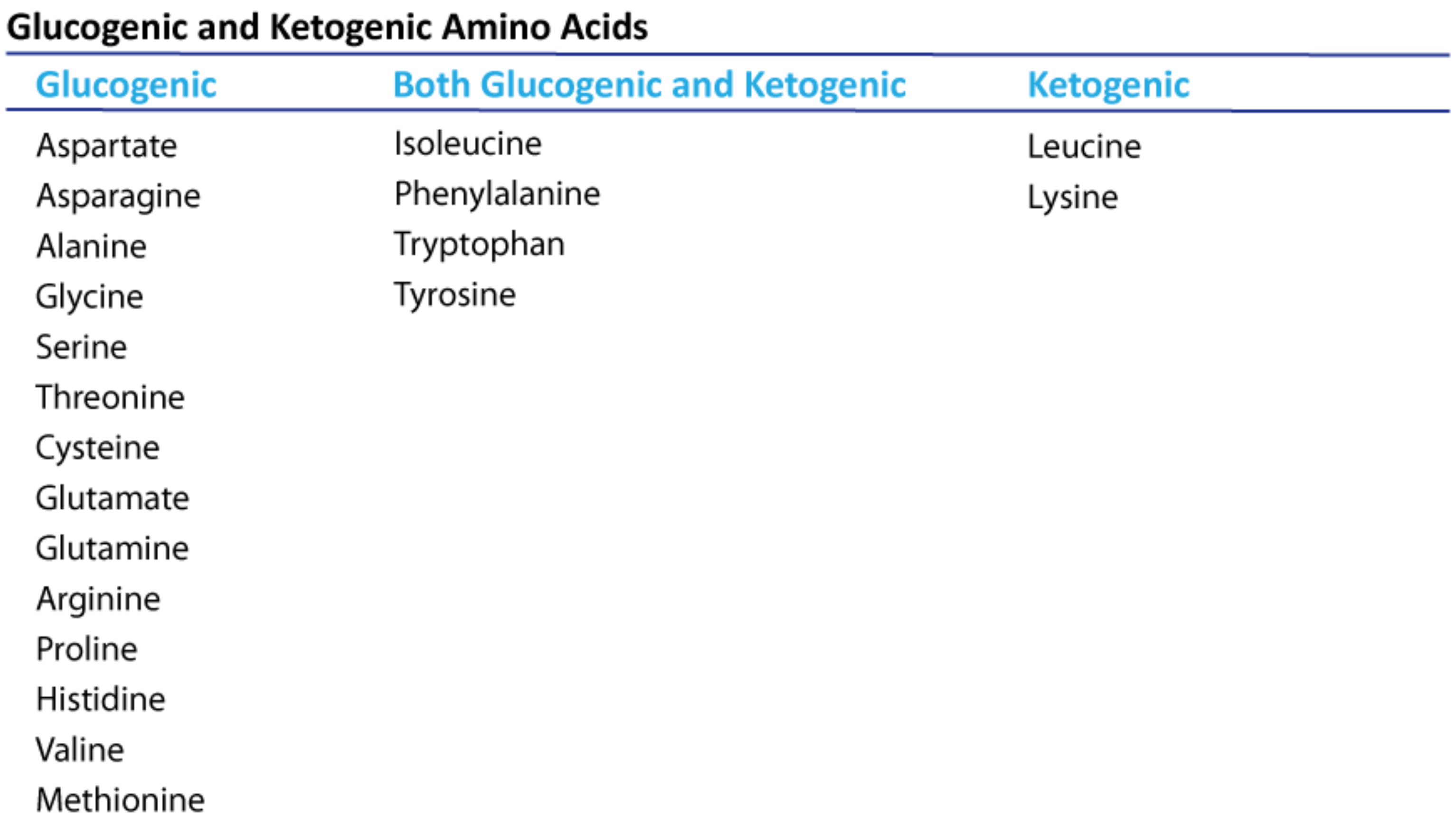
Alpha-ketoacid + amino acid <=> amino acid + alpha-ketoacid

An example reaction follows

Pyruvate + Aspartic acid <=> Alanine + Oxaloacetate

This reaction is catalyzed by an enzyme known as a transaminase. Amino acids, such as glutamate, can also gain nitrogen directly from ammonium ion, as shown below

Alpha-ketoglutarate + NH_4^+ <=> Glutamate



Glucogenic and Ketogenic Amino Acids

Glucogenic	Both Glucogenic and Ketogenic	Ketogenic
Aspartate	Isoleucine	Leucine
Asparagine	Phenylalanine	Lysine
Alanine	Tryptophan	
Glycine	Tyrosine	
Serine		
Threonine		
Cysteine		
Glutamate		
Glutamine		
Arginine		
Proline		
Histidine		
Valine		
Methionine		

This reaction can occur, for example, in nitrifying bacteria, and in places where ammonia waste is produced. Many amino acids can be synthesized from citric acid cycle intermediates. For example, synthesis of the non-essential amino acids occurs as follows: aspartic acid can be made by transamination of oxaloacetate. Glutamate comes from transamination of alpha-ketoglutarate. Pyruvate, as noted, is a precursor of alanine (*via* transamination). Amino acids that can be made from glutamate include glutamine (by addition of an additional ammonium ion), proline, and arginine, Asparagine is made from aspartate by addition of ammonium ion also. Serine is formed from 3-phosphoglycerate and is itself the precursor of both glycine and cysteine. Cysteine and serine are also made from methionine. Tyrosine is made by hydroxylation of phenylalanine.

Amino Acid Catabolism

Breakdown of glutamine by glutaminase is a source of ammonium ion in the cell. The other product is glutamate. Glutamate, of course, can be converted by a transamination reaction to alpha-ketoglutarate, which can be oxidized in the citric acid cycle.

Asparagine can similarly be broken to ammonium and aspartate by **asparaginase** and aspartate can be converted by transamination to oxaloacetate for oxidation in the citric acid cycle.

Alanine is converted to pyruvate in a transamination reaction, making it glucogenic.

Arginine is hydrolzyed in the urea cycle to yield urea and ornithine.

Proline is catabolized to glutamate in a reversal of its synthesis pathway.

Serine donates a carbon to form a folate and the other product of the reaction is glycine, which is itself oxidized to carbon dioxide and ammonia. Glycine can also be converted back to serine, which can also be converted back to 3-phosphoglycerate or pyruvate.

Threonine can be broken down in three pathways, though only two are relevant for humans. One pathway leads to acetyl-CoA and glycine. The other leads to pyruvate.

Cysteine can be broken down in several ways. The simplest occurs in the liver, where a desulfurase can act on it to yield hydrogen sulfide and pyruvate.

Methionine can be converted to cysteine for further metabolism. It can be converted to succinyl-CoA for oxidation in the citric acid cycle. It can also be converted to **S-Adenosyl-Methionine** (SAM), a carbon donor.

Isoleucine and valine can also be converted to succinyl-CoA after conversion first to propionyl-CoA. Since conversion of propionyl-CoA to succinyl-CoA requires vitamin B_{12}, catabolism of these amino acids also requires the vitamin.

Phenylalanine is converted during catabolism to tyrosine, which is degraded ultimately to fumarate and acetoacetate. Thus, both of these amino acids are glucogenic and ketogenic. Tyrosine can also be converted to **dopamine**, **norepinephrine**, and **epinephrine**.

Leucine and lysine can be catabolized to acetoacetate and acetyl-CoA. Lysine is also an important precursor of carnitine.

Histidine can be catabolized by bacteria in intestines to **histamine**, which causes construction or dilation of various blood vessels when in excess.

Tryptophan's catabolism is complex, but can proceed through alanine, acetoacetate and acetyl-CoA

In summary, the following are metabolized to pyruvate – alanine, cysteine, glycine, serine, and threonine

Oxaloacetate is produced from aspartate and asparagine

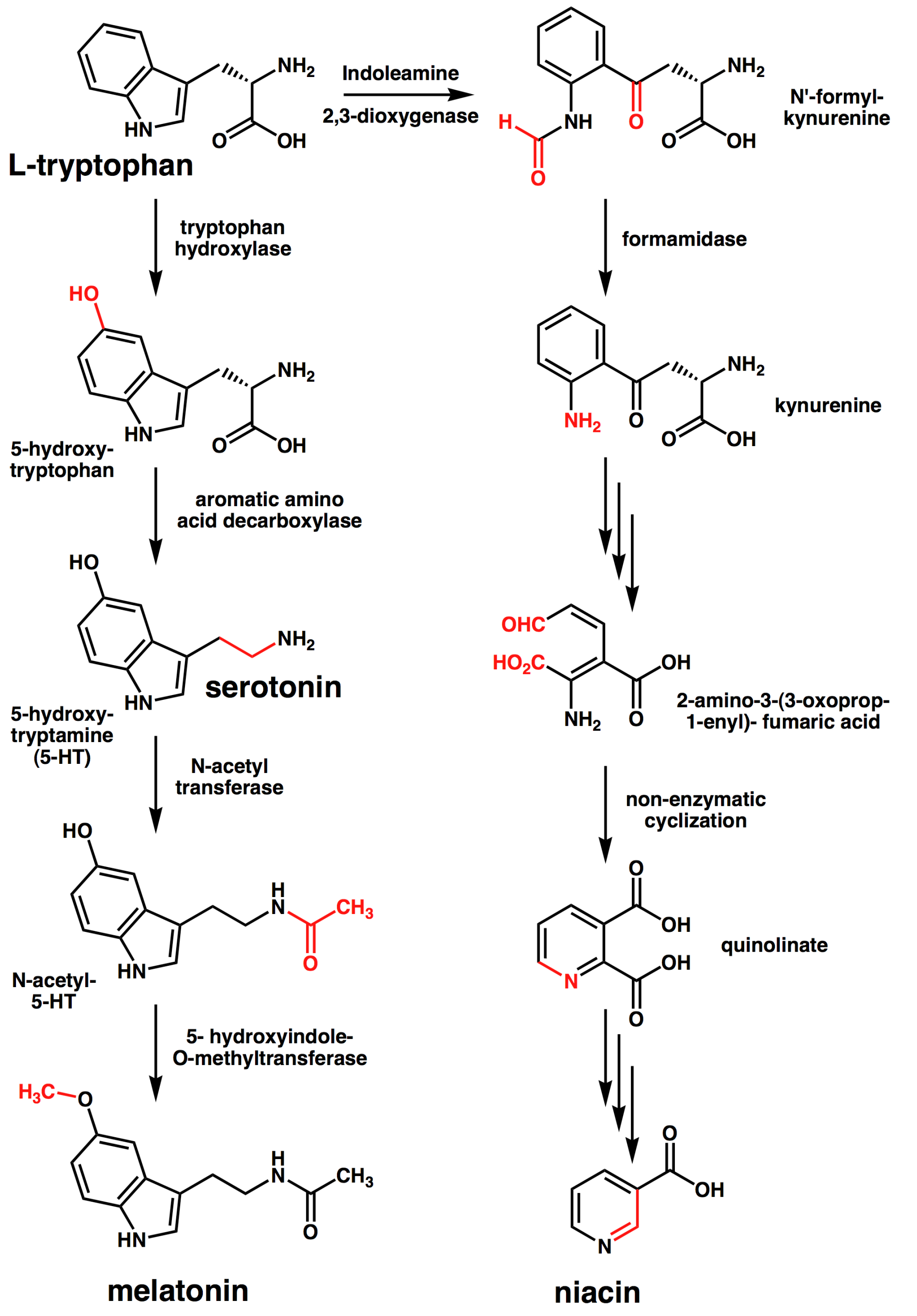
Succinyl-CoA is produced from isoleucine, valine, and methionine

Alpha-ketoglutarate is produced from arginine, glutamate, glutamine, histidine and proline.

Phenylalanine and tyrosine are broken down to fumarate and acetoacetate

Leucine and lysine yield acetoacetate and acetyl-CoA.

Tryptophan leads to alanine, acetoacetate and acetyl-CoA


L-tryptophan
NH$_2$
HN
O
OH
Indoleamine 2,3-dioxygenase
N'-formyl-kynurenine
H
NH
tryptophan hydroxylase
formamidase
HO
5-hydroxy-tryptophan
kynurenine
aromatic amino acid decarboxylase
serotonin
5-hydroxy-tryptamine (5-HT)
OHC
HO$_2$C
2-amino-3-(3-oxoprop-1-enyl)- fumaric acid
N-acetyl transferase
non-enzymatic cyclization
CH$_3$
N-acetyl-5-HT
quinolinate
N
5- hydroxyindole-O-methyltransferase
H$_3$C
melatonin
niacin

Conversion of L-tryptophan into Serotonin, Melatonin, and Niacin.

from Wikipedia

Last, amino acids, besides being incorporated into proteins, serve as precursors of important compounds, including **serotonin** (from tryptophan), porphyrin heme (from glycine), **nitric oxide** (from arginine), and nucleotides (from aspartate, glycine, and glutamine).

Nucleotide Metabolism

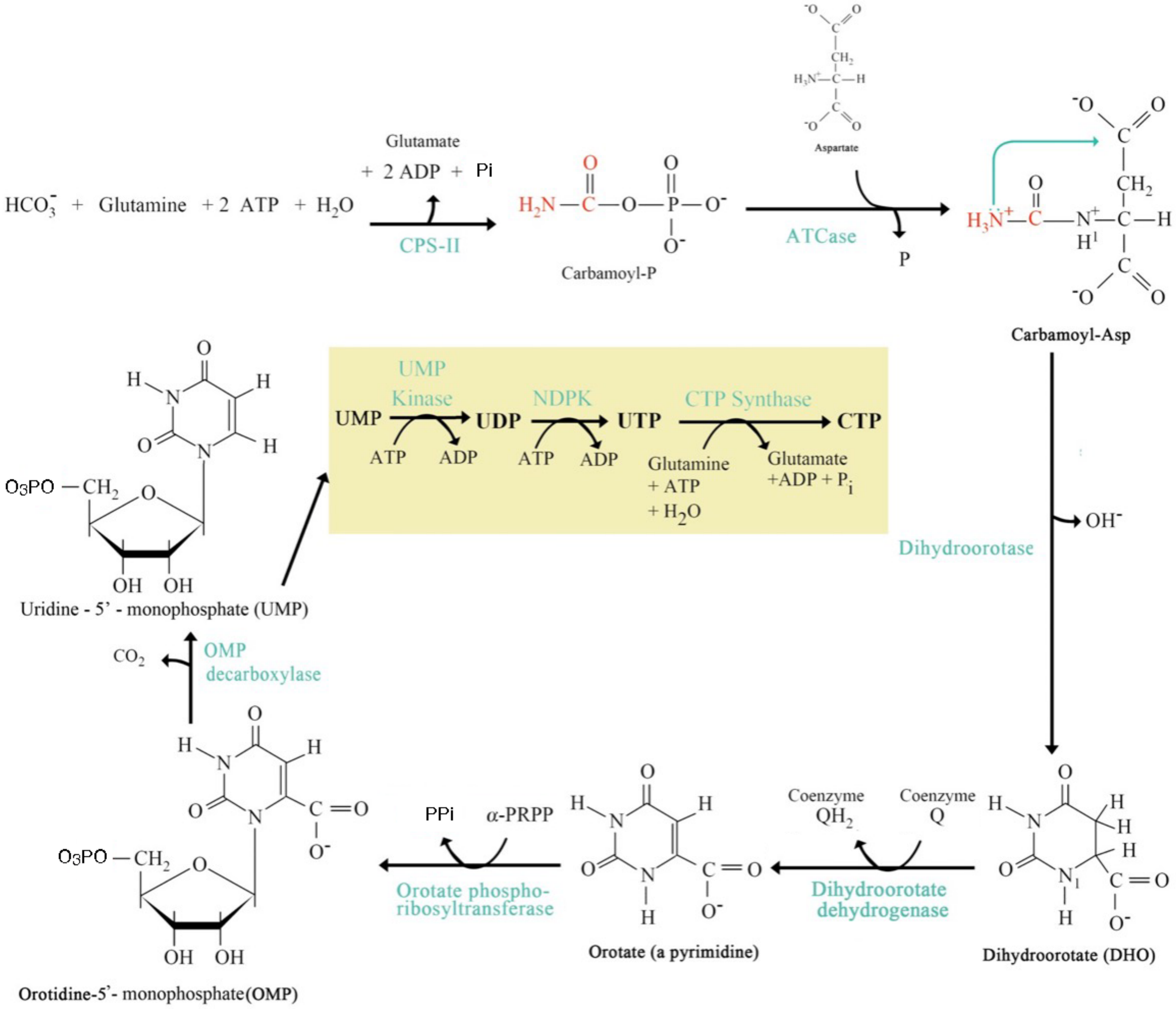
Arguably, the most interesting metabolic pathways from the perspective of regulation are those leading to the synthesis of nucleotides. We shall consider **ribonucleotide** synthesis from from scratch (*de novo* synthesis). **Deoxyribonucleotide** synthesis from ribonucleotides will be considered separately.

Synthesis of ribonucleotides by the *de novo* method occurs in two pathways – one for purines and one for pyrimidines. What is notable about both of these pathways is that nucleotides are built from very simple building blocks.

Pyrimidine *de novo* Biosynthesis

Starting materials for pyrimidine biosynthesis (shown in the figure) include bicarbonate, amine from glutamine, and phosphate from ATP to make **carbamoyl-phosphate** (similar to the reaction of the urea cycle). Joining of carbamoyl phosphate to aspartic acid (forming carbamoyl aspartate) is catalyzed by the most important regulatory enzyme of the cycle, aspartate transcarbamoylase (also called aspartate carbamoyltransferase or ATCase).

ATCase is regulated by three compounds. One of these (aspartate) is a substrate and it activates the enzyme by binding


HCO3- + Glutamine + 2 ATP + H2O
Glutamate + 2 ADP + Pi
CPS-II
Carbamoyl-P
Aspartate
ATCase
P
Carbamoyl-Asp
Dihydroorotase
OH-
Dihydroorotate (DHO)
Coenzyme QH2
Coenzyme Q
Dihydroorotate dehydrogenase
Orotate (a pyrimidine)
PPi
α-PRPP
Orotate phosphoribosyltransferase
Orotidine-5'- monophosphate(OMP)
CO2
OMP decarboxylase
Uridine - 5' - monophosphate (UMP)
UMP Kinase
UMP → UDP
ATP ADP
NDPK
UDP → UTP
ATP ADP
CTP Synthase
UTP → CTP
Glutamine + ATP + H2O
Glutamate +ADP + Pi

De novo Synthesis of Pyrimidine Ribonucleotides to the catalytic site and favoring the enzyme's R state. The other two regulators bind to regulatory subunits of the enzyme and either inhibit (CTP) or activate (ATP) the enzyme.

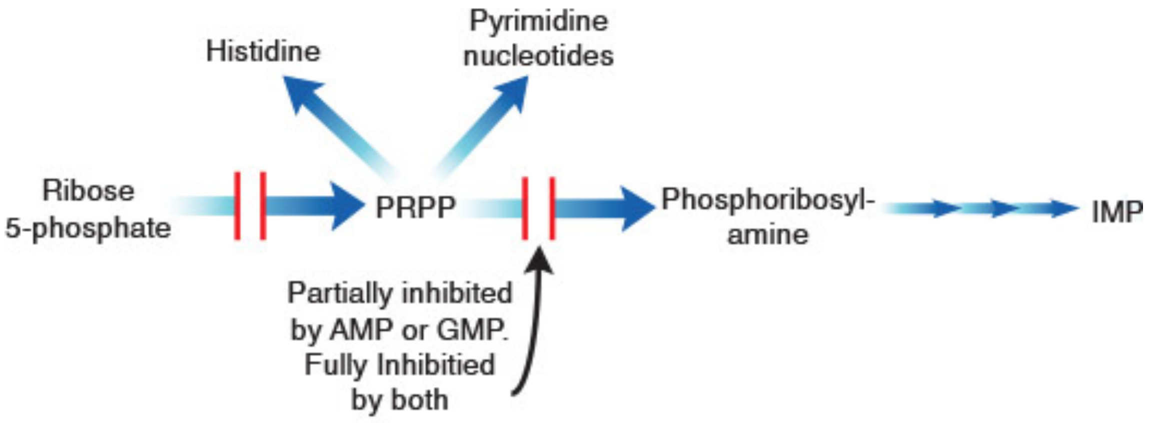
The reaction product, carbamoyl aspartate, is transformed in two reactions to **orotic acid**, which is, in turn combined with **phosphoribosylpyrophosphate** (**PRPP**). The product of that reaction, orotidyl monophosphate (**OMP**) is decarboxylated to form the first pyrimidine nucleotide, **UMP**. Conversion of UMP to **UDP** is catalyzed by **nucleoside monophosphate kinases** (NMPs) and UDP is converted to **UTP** by **nucleoside diphosphokinase** (**NDPK**).

UDP (like all of the nucleoside diphosphates) is a branch point to deoxyribonucleoside diphosphates, catalyzed by ribonucleotide reductases, which are discussed later. UTP is converted to **CTP** by **CTP synthase**. This enzyme, which uses an amino group from glutamine for the reaction, serves to balance the relative amounts of CTP and UTP, thanks to inhibition by excess CTP.

Purine *de novo* Biosynthesis

Synthesis of purine nucleotides differs fundamentally from that of pyrimidine nucleotides in that the bases are built on the ribose ring. The starting material is ribose-5-phosphate, which is phosphorylated by **PRPP synthetase** to PRPP using two phosphates from ATP. **PRPP amidotransferase** catalyzes the transfer of an amine group to PRPP, replacing the **pyrophosphate** on carbon 1. Thus begins the synthesis of the purine ring.

PRPP amidotransferase is regulated partly by **GMP** and partly by **AMP**. The presence of either of these can reduce the enzyme's activity. Only when both are present is the enzyme fully inactivated. Subsequent reactions include adding glycine, adding carbon (from N_{10}-formyltetrahydrofolate), adding amine (from glutamine), closing of the first ring, addition of carboxyl (from CO_2), addition of aspartate, loss of fumarate (a net gain of an amine), addition of another carbon (from N_{10}-formyltetrahydrofolate), and closing of the second ring to form **inosine monophosphate** (**IMP**).

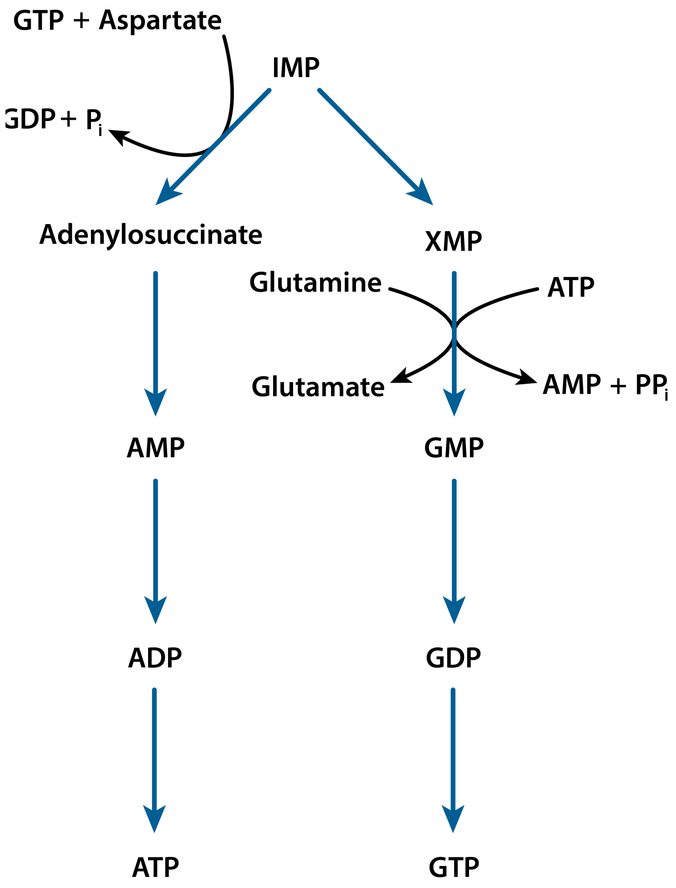


Beginning of Purine Metabolism

See Kevin's Nucleotide Metabolism lectures on YouTube HERE and HERE

IMP is a branch point for the

synthesis of the **adenine** and **guanine** nucleotides. The pathway leading from IMP to AMP involves addition of amine from asparate and requires energy from **GTP**. The pathway from IMP to GMP involves an oxidation and addition of an amine from glutamine. It also requires energy from ATP. The pathway leading to GMP is inhibited by its end product and the pathway to AMP is inhibited by its end product.

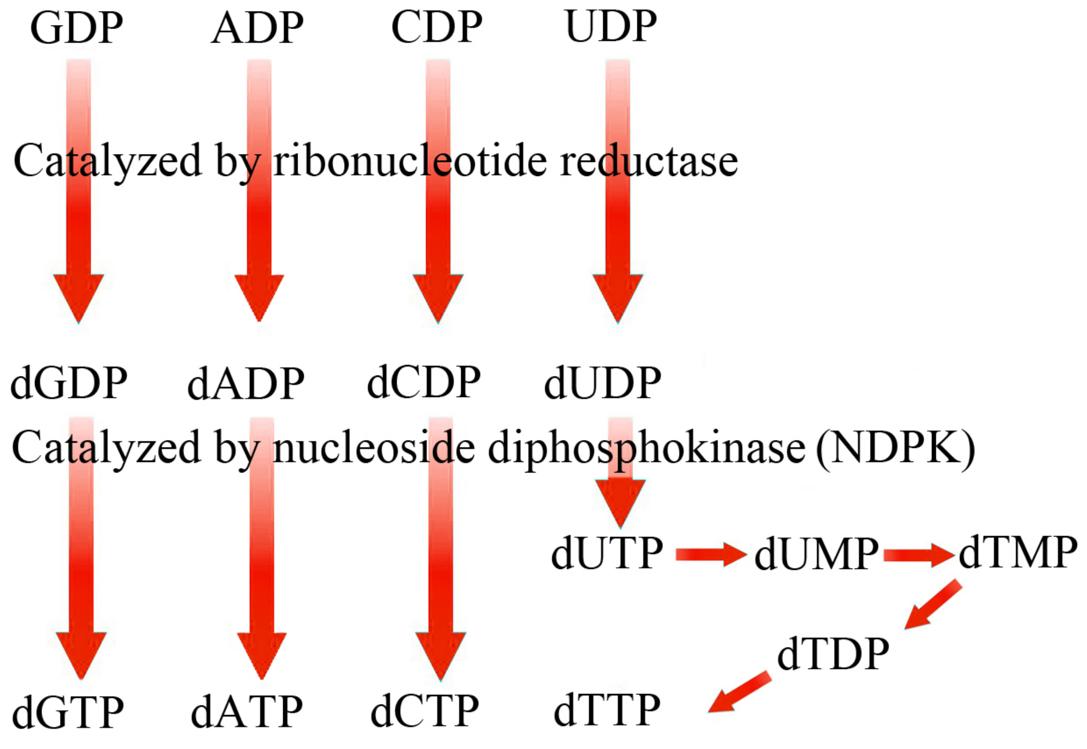

GTP + Aspartate
IMP
GDP + P$_i$
Adenylosuccinate
XMP
Glutamine
ATP
Glutamate
AMP + PP$_i$
AMP
GMP
ADP
GDP
ATP
GTP

Synthesis of ATP and GTP

Thus, balance of the purine nucleotides is achieved from the IMP branch point forward. It is at this point that the significance of the unusual regulation of PRPP amidotransferase becomes apparent. If there is an imbalance of AMP or GMP, the enzyme is slowed, but not stopped, thus allowing the reactions leading to IMP to proceed, albeit slowly. At IMP, the nucleotide in excess feedback inhibits its own synthesis, thus allowing the partner purine nucleotide to be made and balance to be achieved. When both nucleotides are in abundance, then PRPP amidotransferase is fully inhibited and the production of purines is stopped, thus preventing them from over-accumulating.

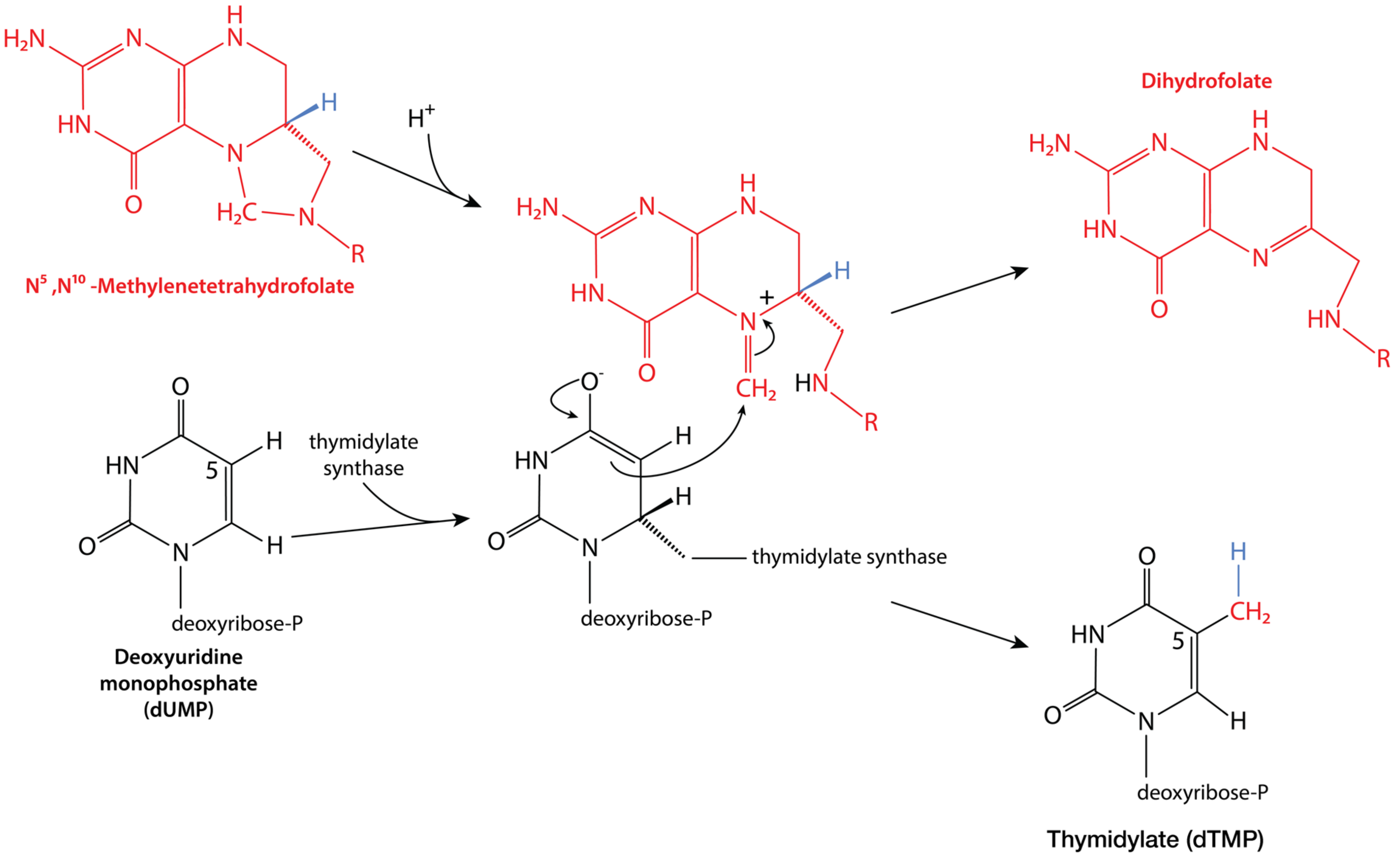
Deoxyribonucleotide *de novo* Synthesis

Synthesis of deoxyribonucleotides *de novo* requires an interesting enzyme called ribonucleotide reductase (RNR). RNR catalyzes the formation of deoxyribonucleotides from ribonucleotides. The most common form of RNR is the Type I enzyme, whose substrates are ribonucleoside diphosphates (ADP, GDP, CDP, or UDP) and the products are deoxyribonucleoside diphosphates


GDP
ADP
CDP
UDP
Catalyzed by ribonucleotide reductase
dGDP
dADP
dCDP
dUDP
Catalyzed by nucleoside diphosphokinase (NDPK)
dUTP
dUMP
dTMP
dTDP
dGTP
dATP
dCTP
dTTP

dNTP *de novo* Synthesis

(dADP, dGDP, dCDP, or dUDP). Thymidine nucleotides are synthesized from dUDP. RNR has two pairs of two identical subunits - R1 (large subunit) and R2 (small subunit). R1 has two allosteric binding sites and an active site. R2 forms a tyrosine radical necessary for the reaction mechanism of the enzyme.


N^5,N^{10}-Methylenetetrahydrofolate
H^+
Dihydrofolate
thymidylate synthase
thymidylate synthase
deoxyribose-P
Deoxyuridine monophosphate (dUMP)
Thymidylate (dTMP)
5

Thymidylate (dTMP) Synthesis

Because a single enzyme, RNR, is responsible for the synthesis of all four deoxyribonucleotides, it is necessary to have mechanisms to ensure that the enzyme produces the correct amounts of each dNDP. This means that the enzyme must be responsive to the levels of the each deoxynucleotide, selectively making more of those that are in short supply, and preventing synthesis of those that are abundant. These demands are met by having two separate control mechanisms, one that determines which substrate will be acted on, and another that controls the enzyme's catalytic activity.

Ribonucleotide reductase is allosterically regulated *via* two binding sites - a *specificity* binding site (binds dNTPs and controls which substrates the enzyme binds and thus, which deoxyribonucleotides are made) and an *activity* binding site (controls whether or not enzyme is active - ATP activates, dATP inactivates).

When a deoxypyrimidine triphosphate, dTTP is abundant, it binds to the specificity site and inhibits binding and reduction of pyrimidine diphosphates (CDP and UDP) but stimulates binding and reduction of GDP by the enzyme. Conversely, binding of the deoxypurine triphosphate, ATP stimulates reduction of pyrimidine diphosphates, CDP and UDP.

Students sometimes confuse the active site of RNR with the activity site. The *active site* is where the reaction is catalyzed, and could also be called the catalytic site, whereas the *activity site* is the allosteric binding site for ATP or dATP that controls whether the enzyme is active.

Synthesis of dTTP by the *de novo* pathway takes a convoluted pathway from dUDP to dUTP to dUMP to dTMP, then dTDP, and finally dTTP. Conversion of dUMP to dTMP, requires a tetrahydrofolate derivative and the enyzme thymidylate synthase. In the process, dihydrofolate is produced and must be converted back to tetrahyrdolate in order to keep nucleotide synthesis occurring. The enzyme involved in the conversion of dihydrofolate to tetrahydrofolate, dihydrofolate reductase (DHFR), is a target of anticancer drugs like methotrexate or aminopterin, which inhibit the enzyme,.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Chapter 8

Signaling

Cells must receive and respond to signals from their surroundings. Cellular signals and the pathways through which they are passed on and amplified to produce the desired effects on their targets are the focus of this section.

Signaling

Cell Signaling

Ligand-gated Ion Channel Receptors

Nuclear Hormone Receptors

G-protein Coupled Receptors (GPCRs)

Receptor Tyrosine Kinases (RTKs)

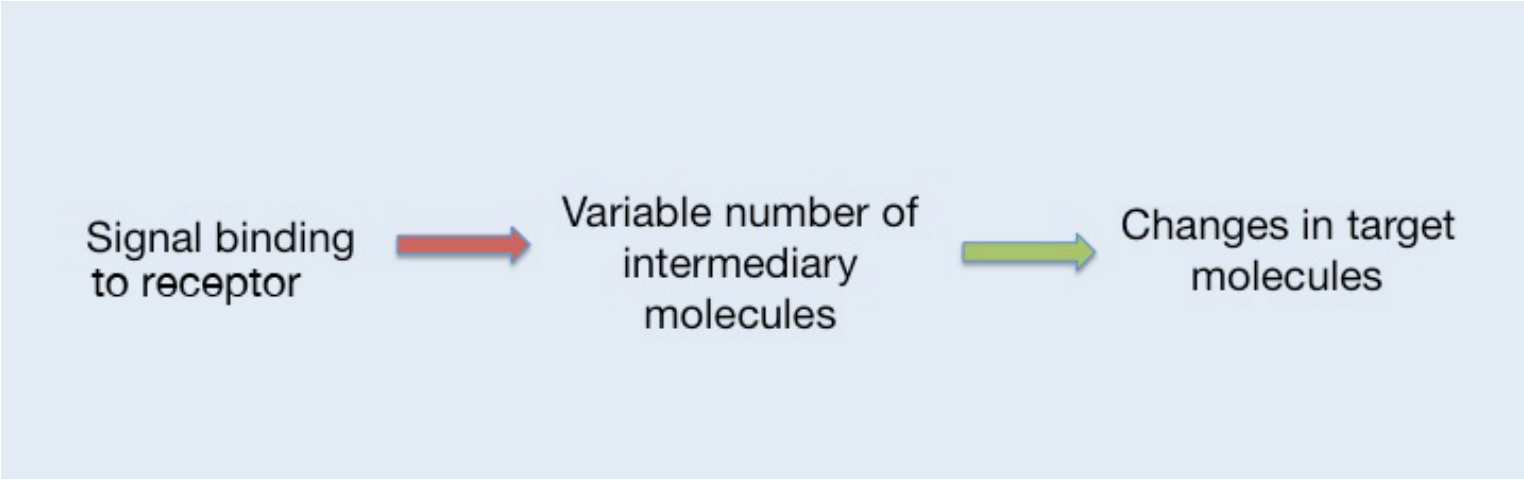
Cell Signaling

How do cells receive signals from their environment and how do they communicate among themselves? It is intuitively obvious that even bacterial cells must be able to sense features of their environment, such as the presence of nutrients or toxins, if they are to survive. In addition to being able to receive information from the environment, multicellular organisms must find ways by which their cells can communicate among themselves. Since different cells take on specialized functions in a multicellular organism, they must be able to coordinate activities perfectly like the musicians in an orchestra performing a complicated piece of music. Cells grow, divide, or differentiate in response to specific signals. They may change shape or migrate to another location. At the physiological level, cells in a multicellular organism, must respond to everything from a meal just eaten to injury, threat or the availability of a mate. They must know when to repair damage to DNA, when to undergo apoptosis (programmed cell death) and even when to regenerate a lost limb. A variety of mechanisms have arisen to ensure that cell-cell communication is not only possible, but astonishingly swift, accurate and reliable.

See Kevin's YouTube lectures on Signaling Mechanisms HERE and HERE

How are signals sent between cells?

Like pretty much everything that happens in cells, signaling is dependent on molecular recognition. The basic principle of cell-cell signaling is simple. A particular kind of molecule, sent by a signaling cell, is recognized and bound by a receptor protein in (or on the surface of) the target cell. The signal molecules are chemically varied- they may be proteins, short peptides, lipids, nucleotides or catecholamines, to name a few. The chemical properties of the signal determine whether its receptors are on the cell surface or intracellular. If the signal is small and hydrophobic it can cross the cell membrane and bind a receptor inside the cell. If, on the other hand, the signal is charged, or very large, it would not be able to diffuse through the plasma membrane. Such signals need receptors on the cell surface, typically transmembrane proteins that have an extracellular portion that binds the signal and an intracellular part that passes on the message within the cell.


Signal binding to receptor
Variable number of intermediary molecules
Changes in target molecules

Cellular Signaling

Receptors are specific for each type of signal, so each cell has many different kinds of receptors that can recognize and bind the many signals it receives. Because different cells have different sets of receptors, they respond to different signals or combinations of signals. The binding of a signal molecule to a receptor sets off a chain of events in the target cell. These events could cause change in various ways, including, but not limited to, alterations in metabolic pathways or gene expression in the target cell.

How the binding of a signal to a receptor brings about change in cells is the topic of this section. Although the specific molecular components of the various signal transduction pathways differ, they all have some features in common:

- The binding of a signal to its receptor is usually, though not always, followed by the generation of a new signal(s) within the cell. The process by which the original signal is converted to a different form and passed on within the cell to bring about change is called signal transduction.

- Most signaling pathways have multiple signal transduction steps by which the signal is relayed through a series of molecular messengers that can amplify and distribute the message to various parts of the cell.

Talking on a Cell Phone

A little cell was waiting for a message from its friend
It couldn't text or Facebook, no e-mails could it send.
What will it do, you wonder, how will it get a clue
About the world around it and what it needs to do?
What languages are spoken by cells that have no voice?
How do they know which way to go when they must make a choice?

Cells use many signals, molecules galore
Arriving at a target cell, some slip in through the door.
Hydrophobic signals through the plasma membrane slide
They're greeted by receptors on the cytoplasmic side.
But if they're hydrophilic on the membrane they must find
A cell-surface receptor to which they soon can bind.

Receptors binding signals will cause a change in cells
Sometimes a gene is turned on, sometimes the message tells
A kinase to phosphorylate and start a big cascade
To nudge awake some enzymes, whose actions can pervade
The metabolic pathways and change the cell's routine
To death or cell division and everything between.

So cells have mechanisms to help communicate
To share a little gossip about their inner state
To tell a friend who's far away an enzyme to secrete
They do this all discreetly without the need to tweet
The chattiest of humans lie silent when in bed
But cells are always talking, unless, of course, they're dead.

Verse by Indira Rajagopal

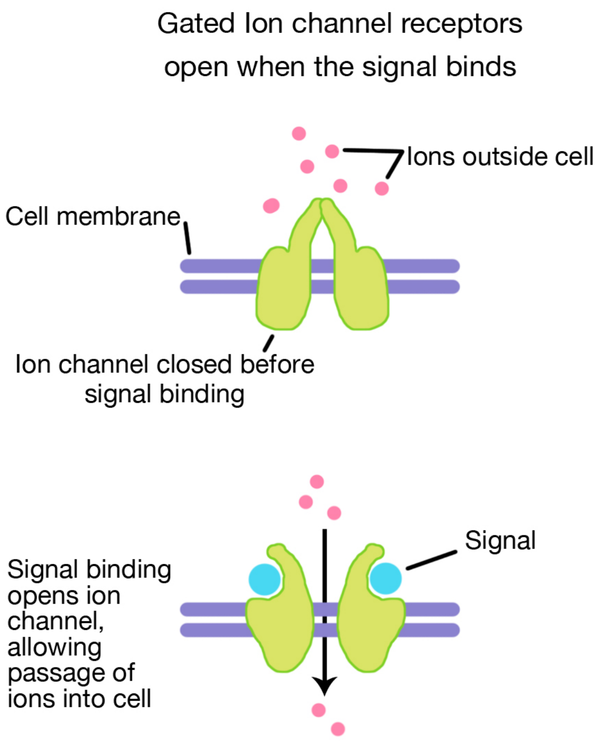
- The last of these messengers usually interacts with a target protein(s) and changes its activity, often by phosphorylation.

When a signal sets a particular pathway in motion, it is acting like an ON switch. This means that once the desired result has been obtained, the cell must have a mechanism that acts as an OFF switch.

Understanding this underlying similarity is helpful, because learning the details of the different pathways becomes merely a matter of identifying which molecular component performs a particular function in each individual case. We will consider several different signal transduction pathways, each mediated by a different kind of receptor. The first two examples we will examine are those with the fewest steps between the binding of the signal by a receptor and a cellular response.

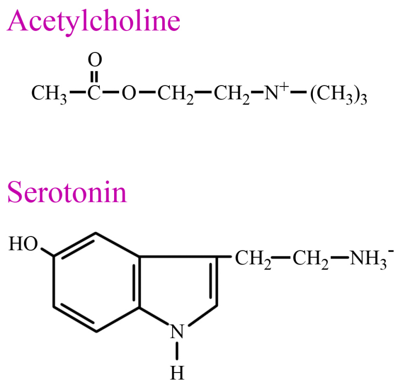
Ligand-gated Ion Channel Receptors

The simplest and fastest of signal pathways is seen in the case of signals whose receptors are gated ion channels. Gated ion channels are made up of multiple transmembrane proteins that create a pore, or channel, in the cell membrane. Depending upon its type, each ion channel is specific to the passage of a particular ionic species. The term "gated" refers to the fact that the ion channel is controlled by a "gate" which must be opened to allow the ions through. The gates are opened by the binding of an incoming signal (ligand) to the receptor, allowing the almost instantaneous passage of millions of ions from one side of the membrane to the other. Changes in the interior environment of the cell are thus brought about in microseconds and in a single step.


Gated Ion channel receptors open when the signal binds
Ions outside cell
Cell membrane
Ion channel closed before signal binding
Signal
Signal binding opens ion channel, allowing passage of ions into cell

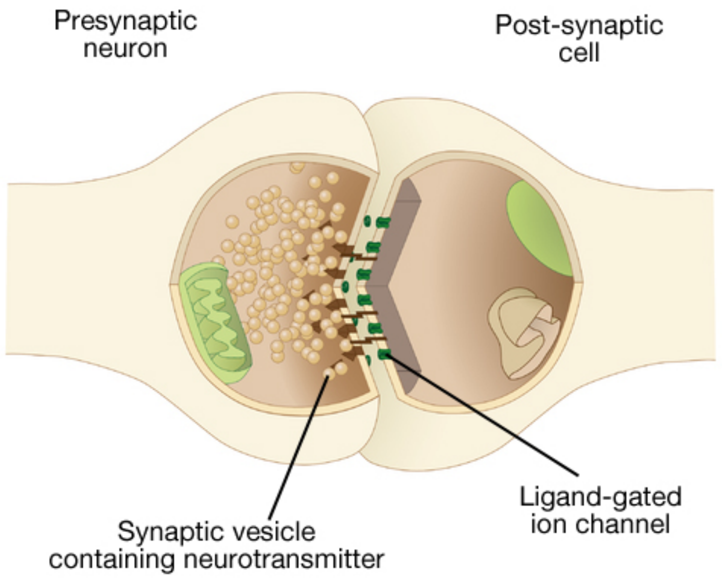
Signaling Through Gated Ion Channels

This type of swift response is seen, for example, in neuromuscular junctions, where muscle cells respond to a message from the neighboring nerve cell. The nerve cell releases a neurotransmitter signal into the synaptic cleft, which is the space between the nerve cell and the muscle cell it is "talking to". Examples of neurotransmitter signal molecules are acetylcholine and serotonin, shown above.


Acetylcholine
Serotonin

Neurotransmitters

When acetylcholine molecules are released into the synaptic cleft (the space between the pre- and post-synaptic cells) they diffuse rapidly till they reach their receptors on the membrane of the muscle cell. The binding of the acetylcholine to its receptor, an ion channel on the membrane of the muscle cell, causes the gate in the ion channel to open. The resulting ion flow through the channel can immediately


Presynaptic neuron
Post-synaptic cell
Synaptic vesicle containing neurotransmitter
Ligand-gated ion channel

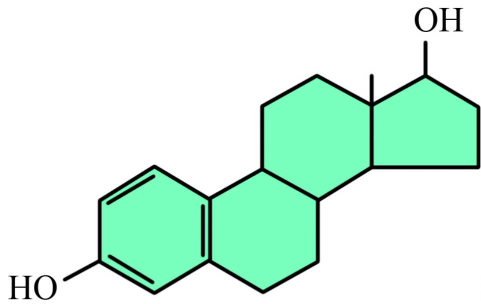
Signaling Across Nerve Cells

From Wikimedia Commons

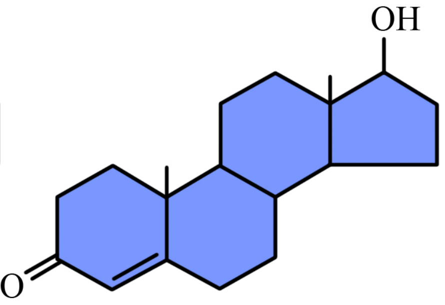
change the membrane potential. This, in turn, can trigger other changes in the cell. The speed with which changes are brought about in neurotransmitter signaling is evident when you think about how quickly you remove your hand from a hot surface. Sensory neurons carry information to the brain from your hand on the hot surface and motor neurons signal to your muscles to move the hand, in less time than it took you to read this sentence!

Nuclear Hormone Receptors

Another type of relatively simple, though much slower, signaling is seen in pathways in which the signals are steroid hormones, like estrogen or testosterone, pictured below. Steroid hormones, as you are aware, are related to cholesterol, and as hydrophobic molecules, they are able to cross the cell membrane by themselves. This is unusual, as most signals coming to cells are incapable of crossing the plasma membrane, and thus, must have cell surface receptors.



Estradiol



Testosterone

By contrast, steroid hormones have receptors inside the cell (intracellular receptors). Steroid hormone receptors are proteins that belong in a family known as the nuclear receptors.

Nuclear hormone receptors are proteins with a double life: they are actually dormant transcription regulators. In the absence of signal, these receptors are in the cytoplasm, complexed with other proteins (HSP in the figure on the following page) and inactive. When a steroid hormone enters the cell, the nuclear hormone receptor binds the hormone and dissociates from the HSP. The receptors, then, with the hormone bound, translocate into the nucleus.

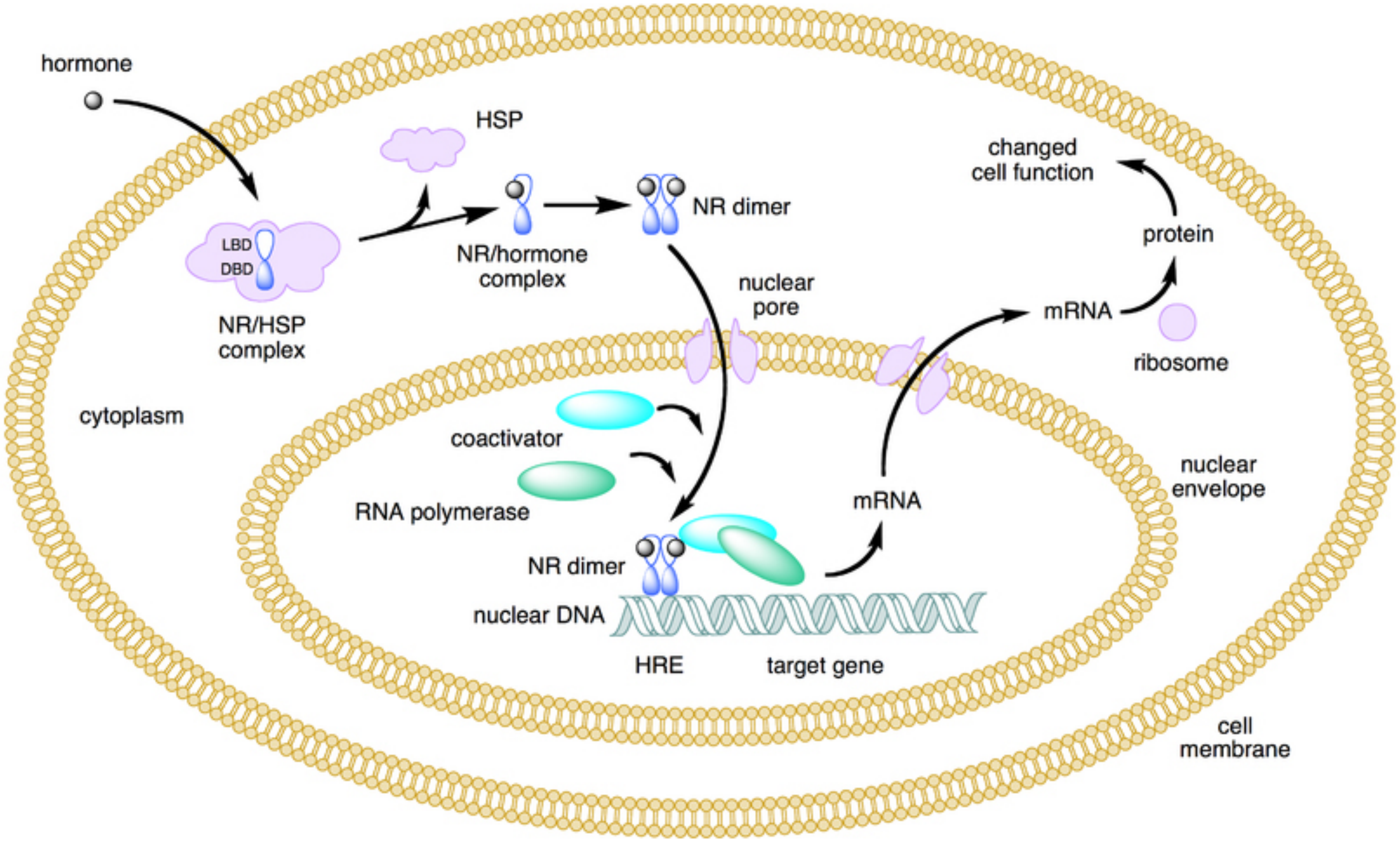
In the nucleus, they regulate the transcription of target genes by binding to their regulatory sequences (labeled HRE for hormone-response elements). The binding of the hormone-receptor complex to the regulatory elements of hormone-responsive genes modulates their expression. Because these responses involve gene expression, they are relatively slow.

See Kevin’s YouTube lectures on Signaling Mechanisms HERE and HERE

Most other signaling pathways, besides the two we have just discussed, involve multiple steps in which the original signal is passed on and amplified through a number of intermediate steps, before the cell responds to the signal.

We will now consider two signaling pathways, each mediated by a major class of cell surface receptor- the G-protein coupled receptors (GPCRs) and the receptor tyrosine kinases (RTKs).

While the specific details of the signaling pathways that follow the binding of signals to each of these receptor types are different, it is easier to learn them when you can see what the pathways have in common, namely, interaction of the signal with a receptor, followed by relaying the signal through a variable number of intermediate molecules, with the last of these molecules interacting with target protein(s) to modify their activity in the cell.


hormone
HSP
LBD
DBD
NR/HSP complex
NR/hormone complex
NR dimer
changed cell function
protein
nuclear pore
mRNA
ribosome
cytoplasm
coactivator
RNA polymerase
mRNA
nuclear envelope
NR dimer
nuclear DNA
HRE
target gene
cell membrane

Steroid Hormones Act by Modulating Expression of Hormone-responsive Genes

G-protein Coupled Receptors (GPCRs)

G-protein coupled receptors are involved in responses of cells to many different kinds of signals, from epinephrine, to odors, to light. In fact, a variety of physiological phenomena including vision, taste, smell and the fight-or-flight response are mediated by GPCRs.

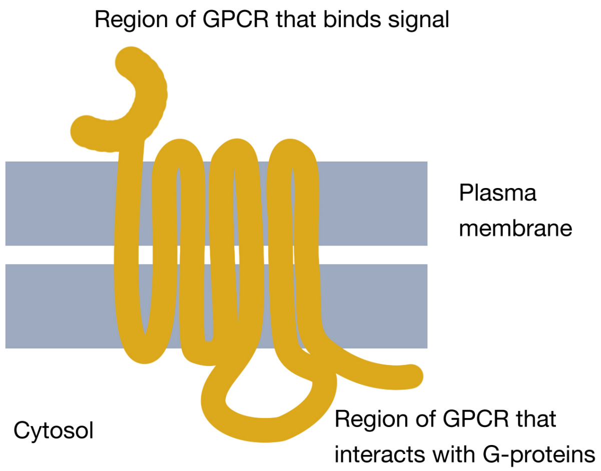
What are G-protein coupled receptors?

G-protein coupled

receptors are cell surface receptors that pass on the signals that they receive with the help of guanine nucleotide binding proteins (a.k.a. G-proteins). Before thinking any further about the signaling pathways downstream of GPCRs, it is necessary to know a few important facts about these receptors and the G-proteins that assist them.

Though there are hundreds of different G-protein coupled receptors, they all have the same basic structure:
they all consist of a single polypeptide chain that threads back and forth seven times through the lipid bilayer of the plasma membrane. For this reason, they are sometimes called seven-pass transmembrane (7TM) receptors.

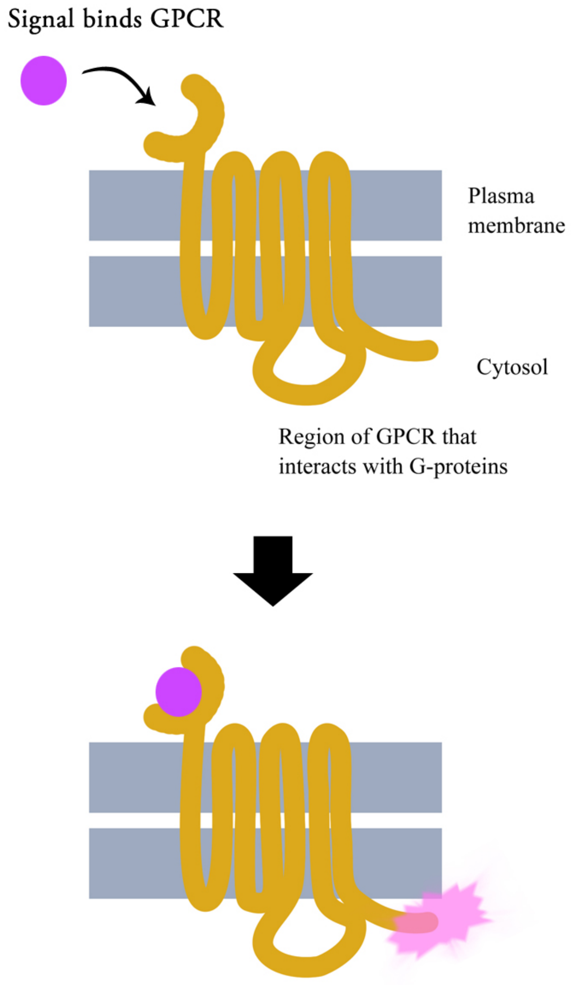
One end of the polypeptide forms the extracellular domain that binds the signal while the other end is in the cytosol of the cell.
When a ligand (signal) binds the extracellular domain of a GPCR, the receptor undergoes a conformational change that allows it to interact with a G-protein that will then pass the signal on to other intermediates in the signaling pathway.


Region of GPCR that binds signal
Plasma membrane
Cytosol
Region of GPCR that interacts with G-proteins

G-protein Coupled Receptor

What is a G-protein?

As noted above, a G-protein is a guanine nucleotide-binding protein that can interact with a G-protein linked receptor. G-proteins are associated with the cytosolic side of the plasma membrane, where they are ideally

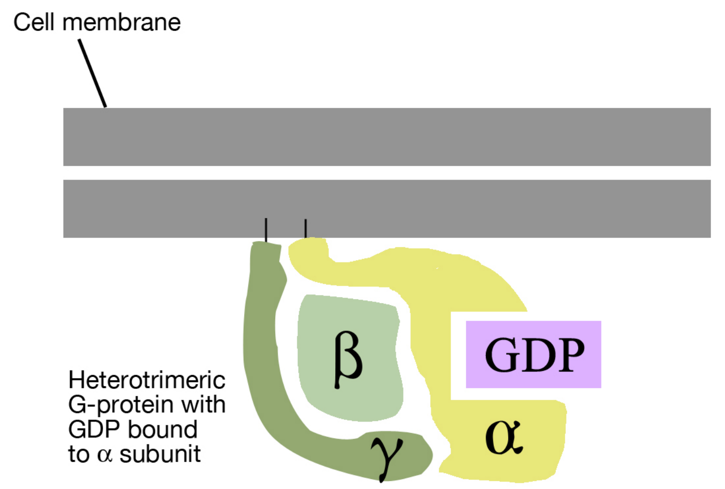

Signal binds GPCR
Plasma membrane
Cytosol
Region of GPCR that interacts with G-proteins

G-protein Coupled Receptor Signaling

situated to interact with the cytosolic tail of the GPCR, when a signal binds to the GPCR.

See Kevin’s YouTube lectures on Signaling Mechanisms HERE and HERE

There are many different G-proteins, all of which share a characteristic structure- they are composed of three subunits called alpha, beta and gamma (αβγ). Because of this, they are sometimes called heterotrimeric G proteins (hetero=different, trimeric= having three parts). The α subunit of such proteins can bind GDP or GTP and is capable of hydrolyzing a GTP molecule bound to it into GDP. In the unstimulated state of the cell, that is, in the absence of a signal bound to the GPCR, the G-proteins are found in the trimeric form (αβγ bound together) and the α subunit has a GDP molecule bound to it.



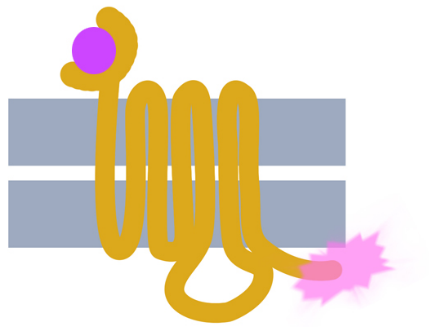
G-protein with GDP Bound

With this background on the structure and general properties of the GPCRs and the G-proteins, we can now look at what happens when a signal arrives at the cell surface and binds to a GPCR.

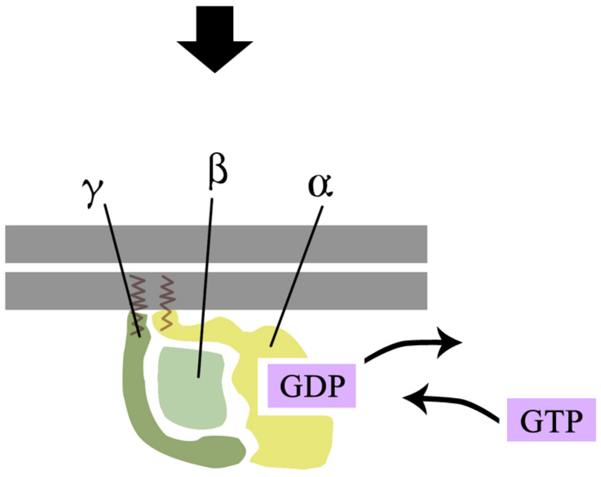
The binding of a signal molecule by the extracellular part of the G-protein linked receptor causes the cytosolic tail of the receptor to interact with, and alter the conformation of, a G-protein. This has two consequences:

- First, the alpha subunit of the G-protein loses its GDP and binds a GTP instead.
- Second, the G-protein breaks up into the GTP-bound α part and the βγ part.

These two parts can diffuse freely along the cytosolic face of the plasma membrane and act upon their targets.



Binding of the signal to GPCR leads to a conformation change in the GPCR’s tail, that results in the activation of a nearby G-protein that exchanges a GDP bound to its α subunit for a GTP.



G-protein Activation

What happens when G-proteins interact with their

target proteins?

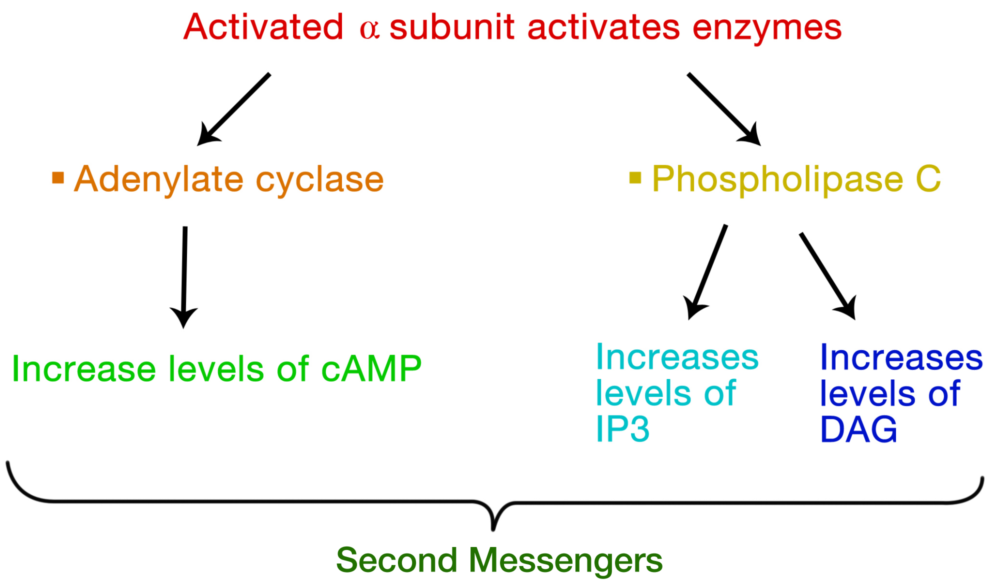
That depends on what the target is. G-proteins interact with different kinds of target proteins, of which we will examine two major categories:

Ion Channels

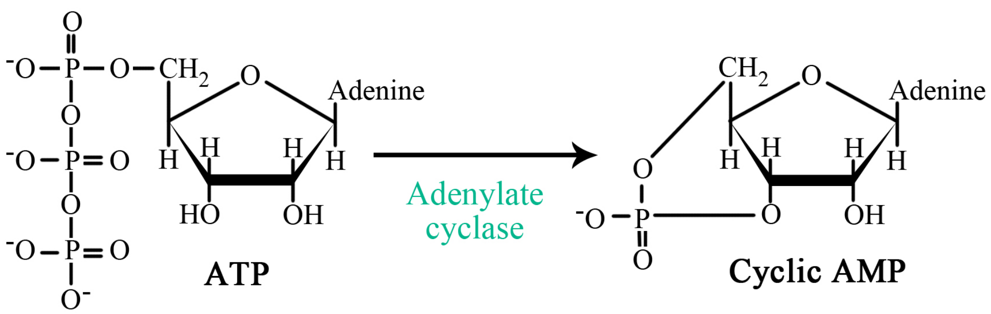
We have earlier seen that some gated ion channels can be opened or closed by the direct binding of neurotransmitters to a receptor that is an ion-channel protein. In other cases, ion channels are regulated by the binding of G-proteins. That is, instead of the signal directly binding to the ion channel, it binds to a GPCR, which activates a G-protein that then binds and opens the ion channel. The change in the distribution of ions across the plasma membrane causes a change in the membrane potential.

Specific Enzymes

The interaction of G-proteins with their target enzymes can regulate the activity of the enzyme, either increasing or decreasing its activity. Often the target enzyme will pass the signal on in another form to another part of the cell. As you might imagine, this kind of response takes a little longer than the kind where an ion channel is opened instantaneously.


Activated α subunit activates enzymes
Adenylate cyclase
Phospholipase C
Increase levels of cAMP
Increases levels of IP3
Increases levels of DAG
Second Messengers

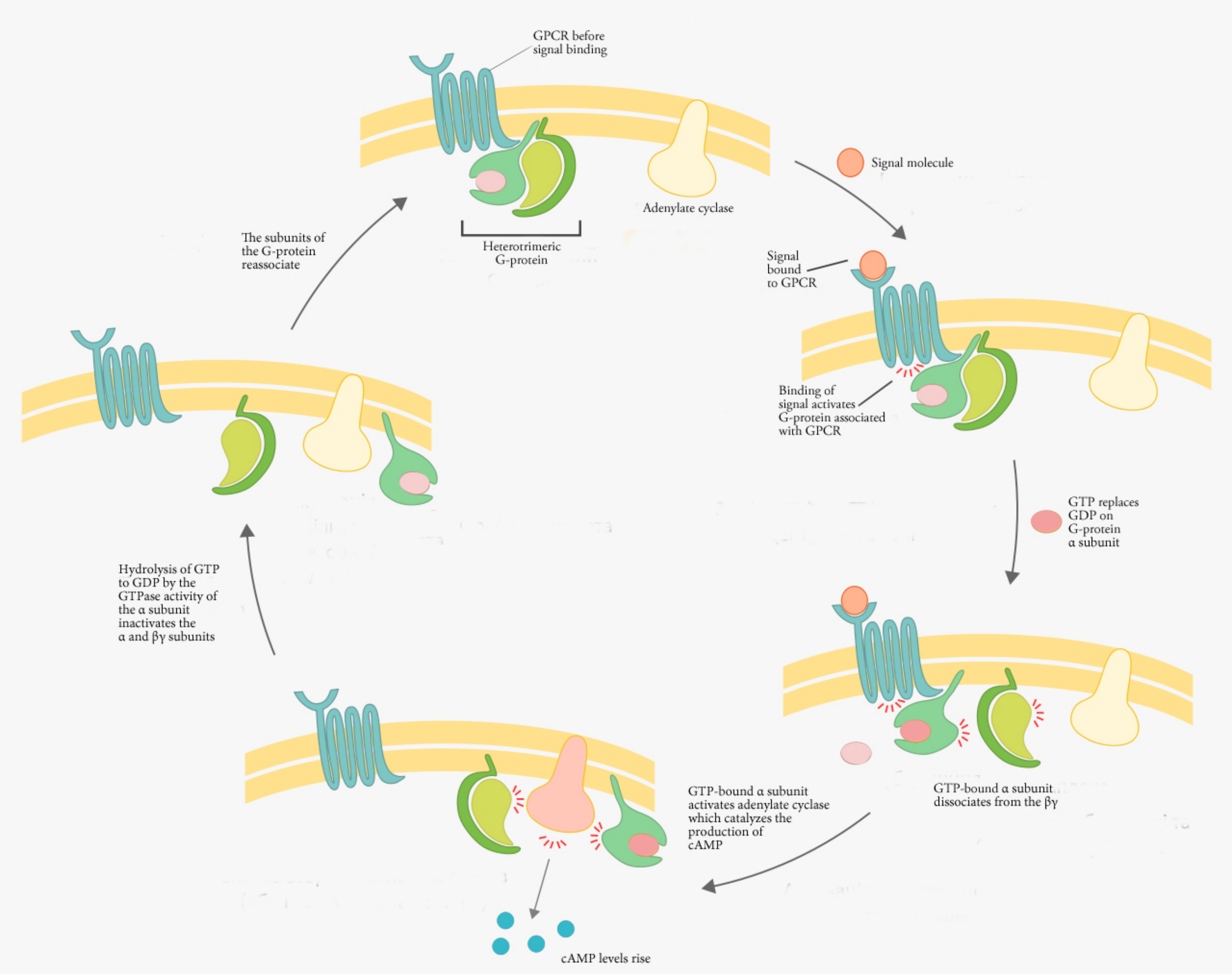
Two well-studied examples of enzymes whose activity is regulated by a G-protein are adenylate cyclase and phospholipase C. When adenylate cyclase is activated, the molecule cAMP is produced in large amounts.


ATP
Adenylate cyclase
Cyclic AMP
Adenine
Adenine

Synthesis of cAMP

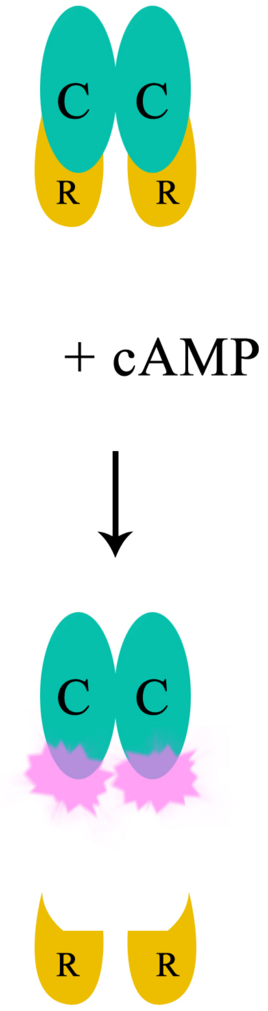
When phospholipase C is activated, the molecules inositol trisphosphate (IP3) and diacylglycerol (DAG) are made. cAMP, IP3 and DAG are second messengers, small, diffusible molecules that can "spread the message" brought by the original signal, to other parts of the cell.

In these cases, the binding of a signal to the GPCR activated a G-protein, which in turn, activated an enzyme that makes a second messenger that can amplify the message in the cell.


GPCR before signal binding
Signal molecule
Adenylate cyclase
Heterotrimeric G-protein
The subunits of the G-protein reassociate
Signal bound to GPCR
Binding of signal activates G-protein associated with GPCR
GTP replaces GDP on G-protein α subunit
Hydrolysis of GTP to GDP by the GTPase activity of the α subunit inactivates the α and βγ subunits
GTP-bound α subunit dissociates from the βγ
GTP-bound α subunit activates adenylate cyclase which catalyzes the production of cAMP
cAMP levels rise

G-protein Signaling Cycle

From Wikimedia Commons


C
C
R
R
+ cAMP
C
C
R
R

Protein Kinase A (PKA) has 2 catalytic subunits and 2 regulatory subunits

cAMP binding to PKA releases catalytic subunits from regulatory subunits, and allows catalytic subunits to phosphorylate their substrates

Protein Kinase A Activation

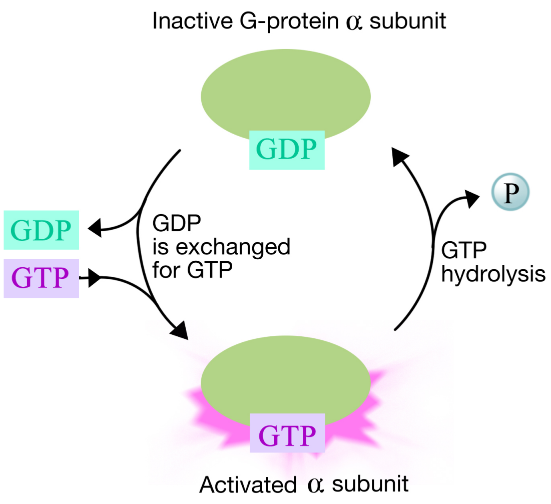
We will first trace the effects of activating adenylate cyclase and the resulting increase in cAMP.

What is the effect of elevated cAMP levels?

cAMP molecules bind to, and activate an enzyme, protein kinase A (PKA). PKA is composed of two catalytic and two regulatory subunits that are bound tightly together. Upon binding of cAMP the catalytic subunits are released from the regulatory subunits,

allowing the enzyme to carry out its function, namely phosphorylating other proteins.

Thus, cAMP can regulate the activity of PKA, which in turn, by phosphorylating other proteins can change their activity. The targets of PKA may be enzymes that are activated by phosphorylation, or they may be proteins that regulate transcription. The phosphorylation of a transcriptional activator, for example, may cause the activator to bind to a regulatory sequence on DNA and to increase the transcription of the gene it controls. The activation of previously inactive enzymes alters the state of the cell by changing the reactions that are occurring within the cell.



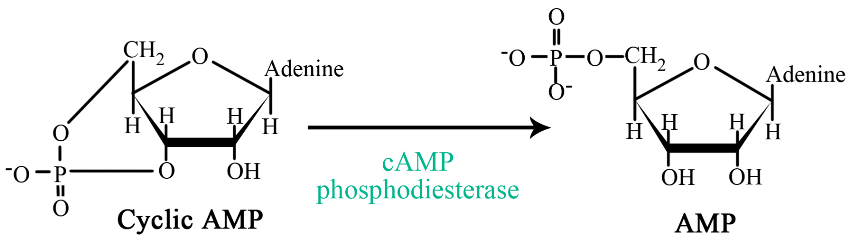
G-protein Nucleotide Swapping

For example, the binding of epinephrine to its receptor on the cell surface, activates, through the action of G-proteins, and subsequent activation of PKA, the phosphorylation of glycogen phosphorylase. The resulting activation of glycogen phosphorylase leads to the breakdown of glycogen, releasing glucose (in the form of glucose-1-phosphate) for use by the cell. Changes in gene expression, likewise, lead to changes in the cell by altering the production of particular proteins in response to the signal.

Although the steps described above seem complicated, they follow the simple pattern outlined at the beginning of this section:

- Binding of signal to receptor
- Several steps where the signal is passed on through intermediate molecules (G-proteins, adenylate cyclase, cAMP, and finally, PKA)
- Phosphorylation of target proteins by the kinase, leading to changes in the cell.

Finally, if the signal binding to the receptor serves as a switch that sets these events in motion, there must be mechanisms to turn the pathway off. The first is at the level of the G-protein. Recall that the alpha subunit of the G-protein is in its free and activated state when it has GTP bound and that it associates with the beta-gamma subunits and has a GDP bound when it is inactive. We also know that the alpha subunit has an activity that enables it to hydrolyze GTP to GDP, as shown in the figure above left. This GTP-hydrolyzing activity makes it possible for the alpha subunit, once it has completed

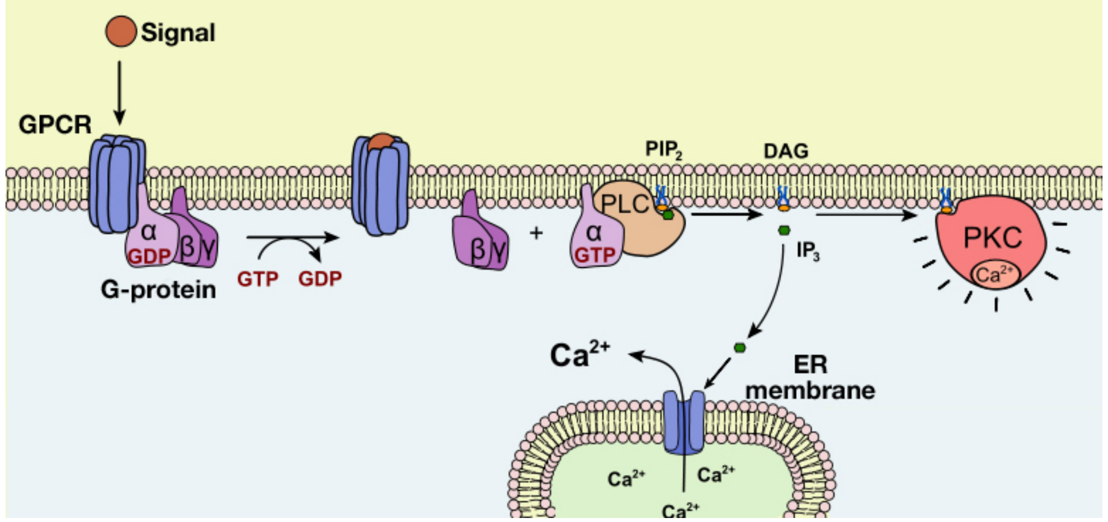


cAMP Breakdown

its task, to return to its GDP bound state, re-associate with the beta-gamma part and become inactive again.

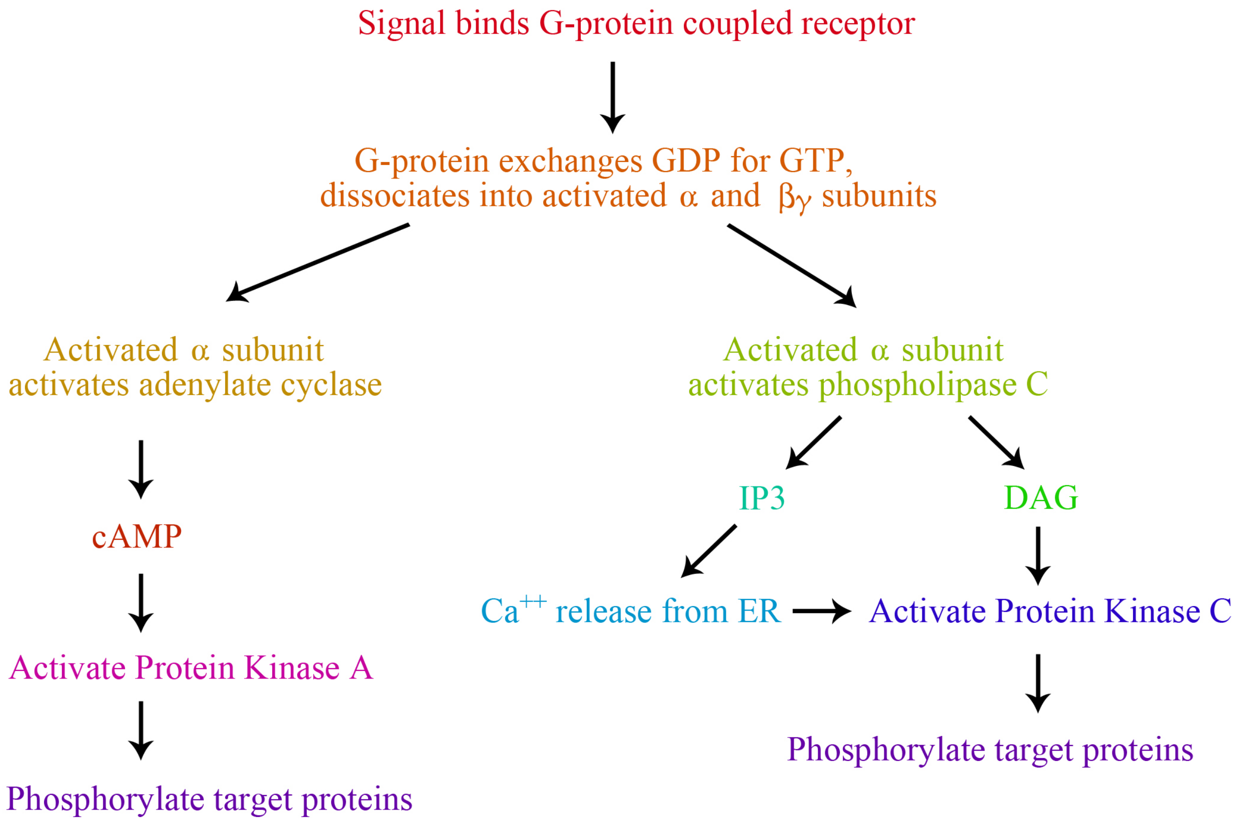
The second "off switch" is further down the signaling pathway, and controls the level of cAMP. We just noted that cAMP levels increase when adenylate cyclase is activated. When its job is done, cAMP is broken down by an enzyme called phosphodiesterase. When cAMP levels drop, PKA returns to its inactive state, putting a halt to the changes brought about by the activation of adenylate cyclase by an activated G-protein.

Let us now examine the events that follow the activation of Phospholipase C (PLC) by a G-protein. As we noted earlier, the activation of PLC results in the production of the second messengers IP_3 and DAG. What do these molecules do?


Signal
GPCR
α
GDP
βγ
G-protein
GTP
GDP
PIP$_2$
DAG
βγ
+
α
GTP
PLC
IP$_3$
PKC
Ca^{2+}
Ca^{2+}
ER membrane
Ca^{2+}
Ca^{2+}
Ca^{2+}

Phospholipase C Signaling

From Wikimedia Commons


Signal binds G-protein coupled receptor
G-protein exchanges GDP for GTP, dissociates into activated α and βγ subunits
Activated α subunit activates adenylate cyclase
cAMP
Activate Protein Kinase A
Phosphorylate target proteins
Activated α subunit activates phospholipase C
IP3
DAG
Ca^{++} release from ER
Activate Protein Kinase C
Phosphorylate target proteins

Signaling Outcomes

The IP_3 and DAG produced by activated phospholipase C work together to activate a protein kinase. First, IP_3 diffuses to the endoplasmic reticulum membrane where it binds to gated calcium ion channels. This causes calcium channels in the ER membrane to open and release large amounts of calcium into the cytoplasm from the ER lumen, as shown in the figure below.

See Kevin’s YouTube lectures on Signaling Mechanisms HERE and HERE

The increase in cytosolic calcium ion concentration has various effects, one of which is to activate a protein kinase called protein kinase C (C for calcium), together with the DAG made in the earlier step. Like PKA, Protein kinase C phosphorylates a variety of proteins in the cell, altering their activity and thus changing the state of the cell.

The pathways leading to PKC and PKA activation following the binding of a signal to a GPCR are summarized above.

Receptor Tyrosine Kinases

Receptor tyrosine kinases mediate responses to a large number of signals, including peptide hormones like insulin and growth factors like epidermal growth factor.

Gee, I Wish I Could Do That

GPCRs, as you know,
Have a G-protein in tow.
Alpha, beta, gamma, sit
Waiting till the signals hit.

When receptors signals bind,
G-proteins respond in kind,
Swapping out their GDP
For triphosphate gleefully.

Alpha, with its GTP,
Leaves all full of energy.
A cyclase it will activate
That acts upon adenylate.

Cyclic AMPs then find
A PKA that they can bind.
With binding of cAMPs
R subunits part from Cs.

C subunits, floating free,
Are on a phosphate adding spree.
PKA phosphorylates
And target proteins activates

Another enzyme, PLC,
Awaits an alpha-GTP
To activate it, so it splits
PIP2, and makes two bits.

IP3 and DAG
Are second messengers, you see.
They work together as a team,
They've got a cunning little scheme.

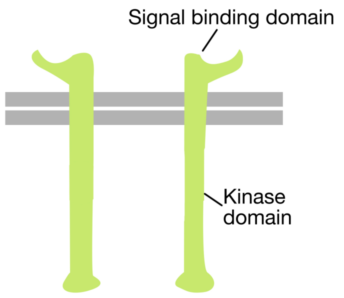
IP3 at ion gates
On ER membranes lets a spate
Of calcium into cytosol
That's bad enough, but that's not all.

The calcium binds a PKC,
And with the help of DAG,
The kinase it can activate
Proteins to phosphorylate.

The binding of a signal small
To GPCRs, caused this all.
Transducing pathways work a spell
And change the actions of a cell.

Verse by Indira Rajagopal

Like the GPCRs, receptor tyrosine kinases bind a signal, then pass the message on through a series of intracellular molecules, the last of which acts on target proteins to change the state of the cell.

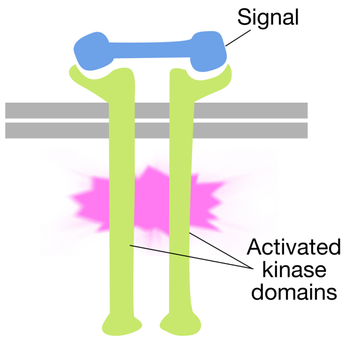


Receptor Tyrosine Kinase before signal binding

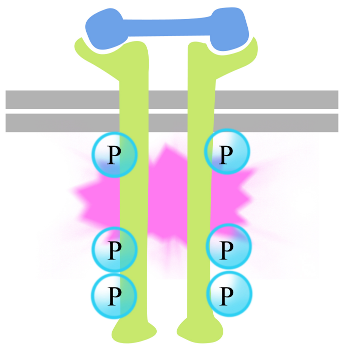
As the name suggests, a receptor tyrosine kinase is a cell surface receptor that also has a tyrosine kinase activity. The signal binding domain of the receptor tyrosine kinase is on the cell surface, while the tyrosine kinase enzymatic activity resides in the cytoplasmic part of the protein (see figure above). A transmembrane alpha helix connects these two regions of the receptor.

What happens when signal molecules bind to receptor tyrosine kinases?

Binding of signal molecules to the extracellular domains of receptor tyrosine kinase molecules causes two receptor molecules to dimerize (come together and associate). This brings the cytoplasmic tails of the receptors close to each other and causes the tyrosine kinase activity of these tails to be turned on. The activated tails then phosphorylate each other on several tyrosine residues. This is called autophosphorylation.



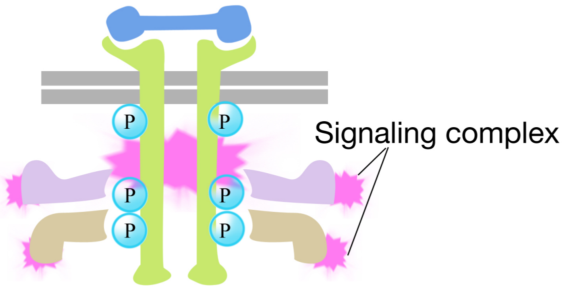
Signal binding causes dimerization of receptor and activation of tyrosine kinase domains



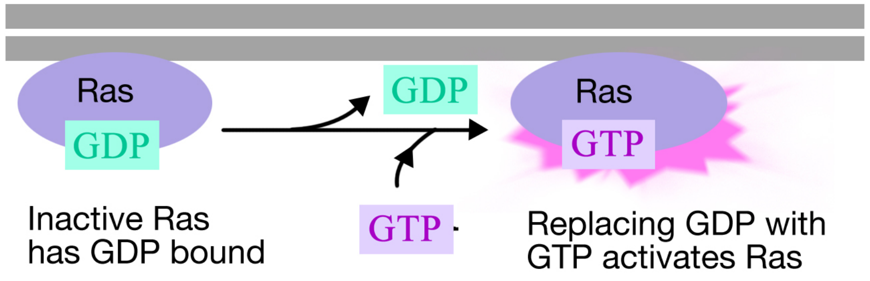
Activated tyrosine kinase domains add phosphate onto each other

The phosphorylation of tyrosines on the receptor tails triggers the assembly of an intracellular signaling complex on the tails. The newly phosphorylated tyrosines serve as binding sites for signaling proteins that then pass the message on to yet other proteins. An important protein that is subsequently activated by the signaling complexes on the receptor tyrosine kinases is called Ras.

The Ras protein is a monomeric guanine nucleotide binding protein that is associated with the cytosolic face of the plasma membrane (in fact, it is a lot like the alpha subunit of trimeric G-proteins). Just like the alpha subunit of a G-protein, Ras is active



Complex of signaling proteins assembles on phosphorylated RTK tails. This complex can activate Ras.


Ras
GDP
GDP
Ras
GTP
GTP
Inactive Ras has GDP bound
Replacing GDP with GTP activates Ras

Ras Activation

when GTP is bound to it and inactive when GDP is bound to it. Also, like the alpha subunit, Ras can hydrolyze the GTP to GDP.

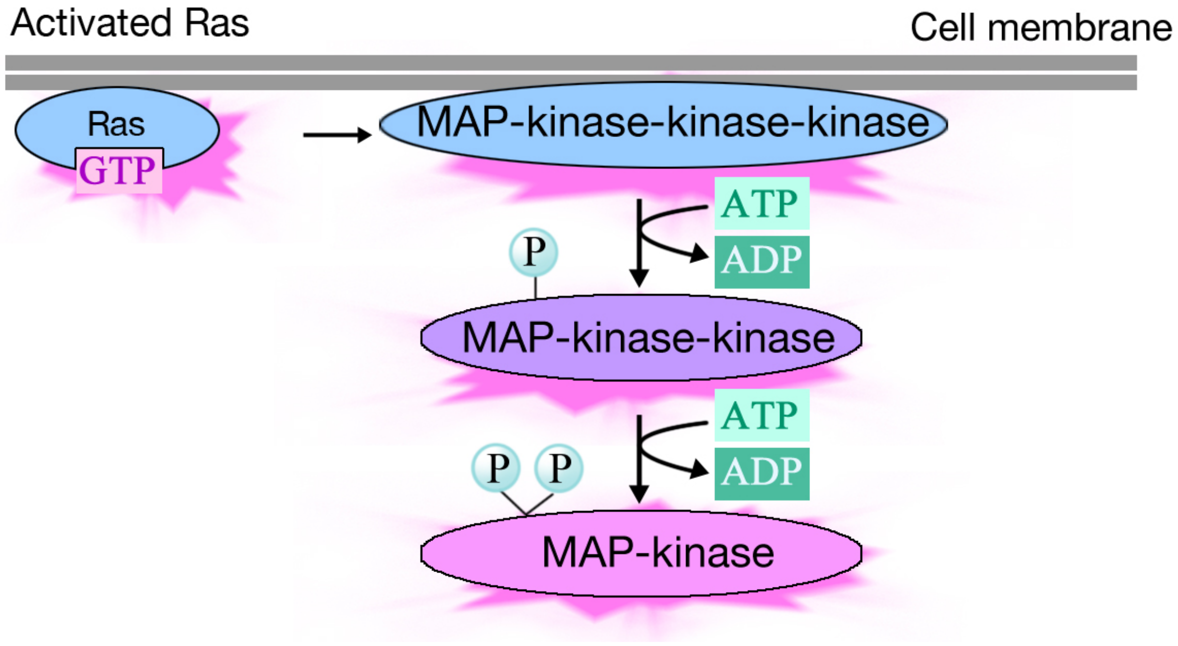
When a signal arrives at the receptor tyrosine kinase, the receptor monomers come together and phosphorylate each others' tyrosines, triggering the assembly of a complex of proteins on the cytoplasmic tail of the receptor. One of the proteins in this complex interacts with Ras and stimulates the exchange of the GDP bound to the inactive Ras for a GTP. This activates the Ras.

Activated Ras triggers a phosphorylation cascade of three protein kinases, which relay and distribute the signal. These protein kinases are members of a group called the MAP kinases (Mitogen Activated Protein Kinases). The final kinase in this cascade phosphorylates various target proteins, including enzymes and transcriptional activators that regulate gene expression.

The phosphorylation of various enzymes can alter their activities, and set off new chemical reactions in the cell, while the phosphorylation of transcriptional activators can change which genes are expressed. The combined effect of changes in gene expression and protein activity alter the cell's physiological state.

Once again, in following the path of signal transduction mediated by RTKs, it is possible to discern the same basic pattern of events: a signal is bound by the extracellular domains of receptor tyrosine kinases, resulting in receptor dimerization and autophosphorylation of the cytosolic tails, thus conveying the message to the interior of the cell.

The message is passed on *via* a signalling complex to Ras which then stimulates a series of kinases. The terminal kinase in the cascade acts on target proteins and brings about in changes in protein activities and gene expression.



Activated Ras Cascade

The descriptions above provide a very simple sketch of some of the major classes of receptors and deal primarily with the mechanistic details of the steps by which signals received by various types of receptors bring about changes in cells. A major take-home lesson is the essential similarity of the different pathways.

Another point to keep in mind is that while we have looked at each individual pathway in isolation, a cell, at any given time receives multiple signals that set off a variety of different responses at once. The pathways described above show a considerable degree of "cross-talk" and the response to any given signal is affected by the other signals that the cell receives simultaneously. The multitude of different receptors, signals and the combinations thereof are the means by which cells are able to respond to an enormous variety of different circumstances.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

The Tao of Hormones

To the tune of "*The Sound of Silence*"

Biochemistry my friend
It's time to study you again
Mechanisms that I need to know
Are the things that really stress me so
"Get these pathways planted firmly in your head,"
Ahern said

Let's start with ep-inephrine
Membrane proteins are well known
Changed on binding this hormone
Rearranging selves without protest
Stimulating a G alpha S
To go open up and displace its GDP
With GTP

Because of ep-inephrine
Active G then moves a ways
Stimulating ad cyclase
So a bunch of cyclic AMP
Binds to kinase and then sets it free
All the active sites of the kinases await
Triphosphate

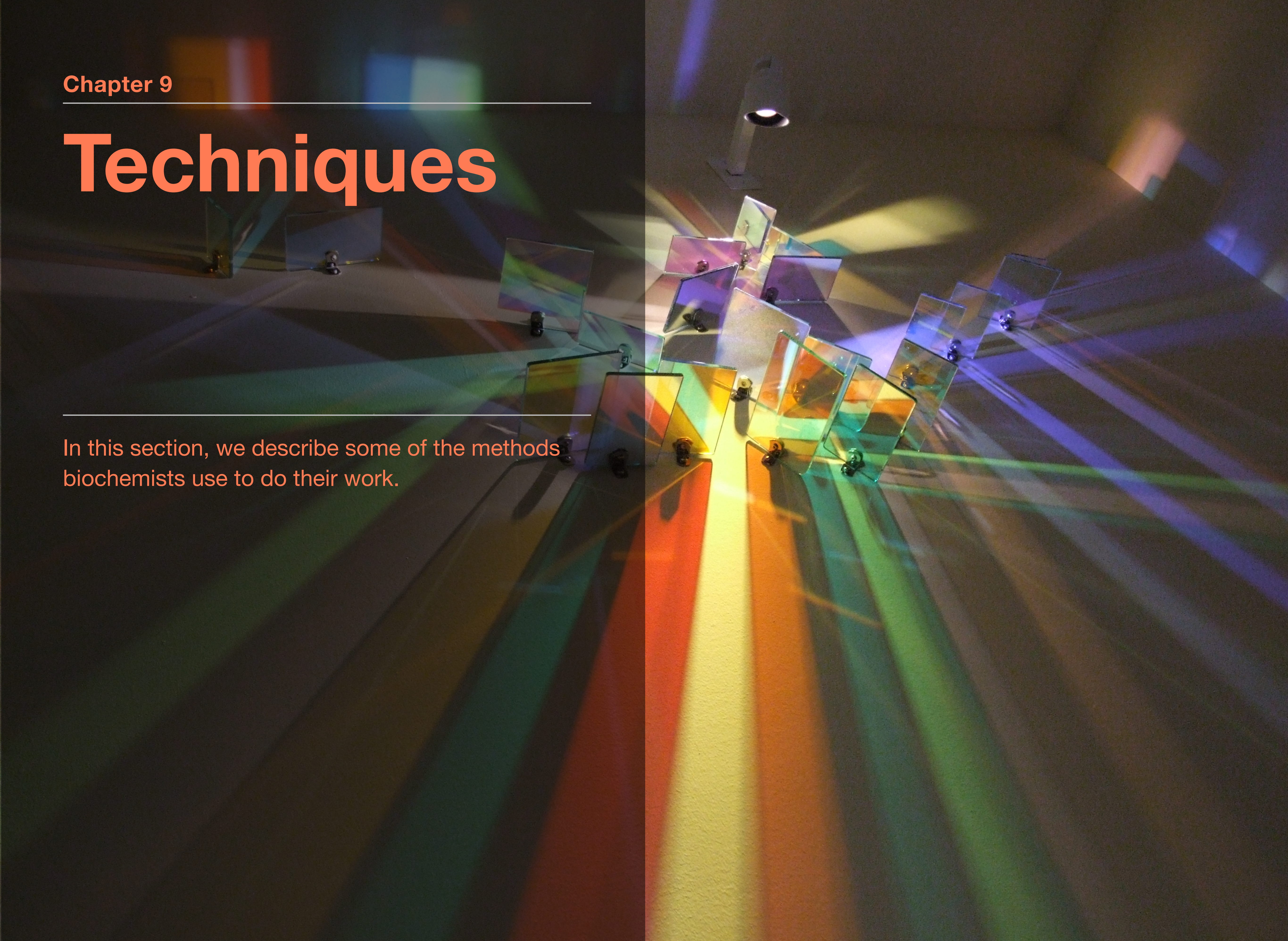
Because of ep-inephrine
Muscles are affected then
Breaking down their glycogen
So they get a wad of energy
In the form of lots of G-1-P
And the synthases that could make a glucose chain
All refrain

Because of ep-inephrine
Now I've reached the pathway end
Going from adrenalin
Here's a trick I learned to get it right
Linking memory to flight or fright
So the mechanism that's the source of anxious fears
Reappears
When I make ep-inephrine

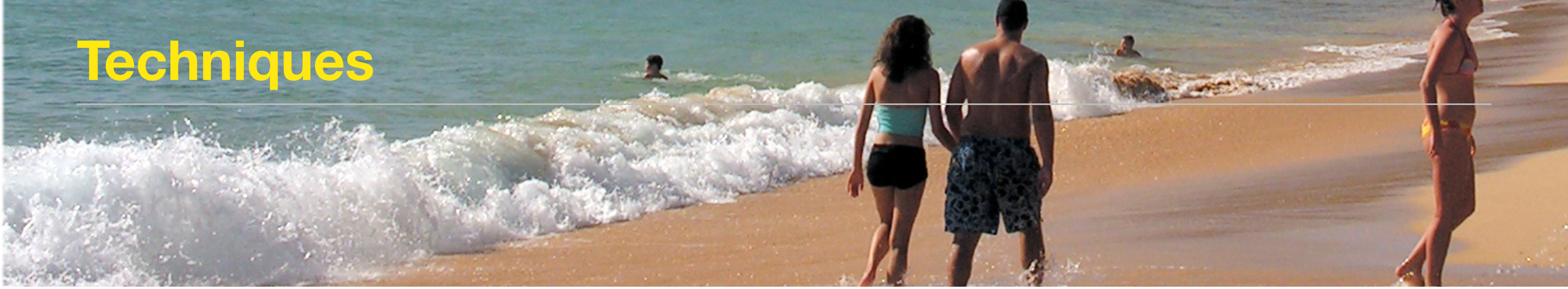
Recorded by Tim Karplus
Lyrics by Kevin Ahern

Chapter 9

Techniques



In this section, we describe some of the methods biochemists use to do their work.



Techniques

- introduction
- Cell Disruption
- Fractionation
- Ion Exchange Chromatography
- Gel Exclusion Chromatography
- Affinity Chromatography
- HPLC
- Histidiine Tagging
- Electrophoresis
    - Agarose
    - SDS-PAGE
    - Isoelectric Focusing
    - 2-D Gel Electrophoresis
- Protein Cleavage
- Microarrays
- Blotting
- Making Recombinant DNAs
- PCR
- *Lac Z* Blue-White Screening
- Reverse Transcription

Introduction

The environment of a cell is very complex, making it very difficult, if not impossible, to study individual reactions, enzymes, or pathways within it. For this reason, biochemists prefer to isolate molecules (enzymes, DNAs, RNAs, and other molecules of interest) so they can be analyzed without interference from the millions of other processes occurring simultaneously in the cell. Many of the methods used in isolating molecules from cells involve some form of chromatography. To separate compounds from their cellular environments, one must first break open (lyse) the cells.

Cell Disruption

There are several ways to break open cells. Lysis methods include lowering the ionic strength of the medium cells are kept in. This can cause cells to swell and burst. Mild surfactants may be used to enhance the efficiency of lysis. Most bacteria, yeast, and plant tissues, which have cell walls, are resistant to such osmotic shocks, however, and stronger disruption techniques are often required.

Click HERE and HERE, for Kevin's Techniques lectures on YouTube

Enzymes may be useful in helping to degrade the cell walls. Lysozyme, for example, is very useful for breaking down bacterial walls. Other enzymes commonly employed include cellulase (plants), glycanases, proteases, mannases, and others.

Mechanical agitation may be employed in the form of tiny beads that are shaken with a suspension of cells. As the beads bombard the cells at high speed, they break them open. Sonication (20-50 kHz sound waves) provides an alternative method for lysing cells. The method is noisy, however, and generates heat that can be problematic for heat-sensitive compounds.

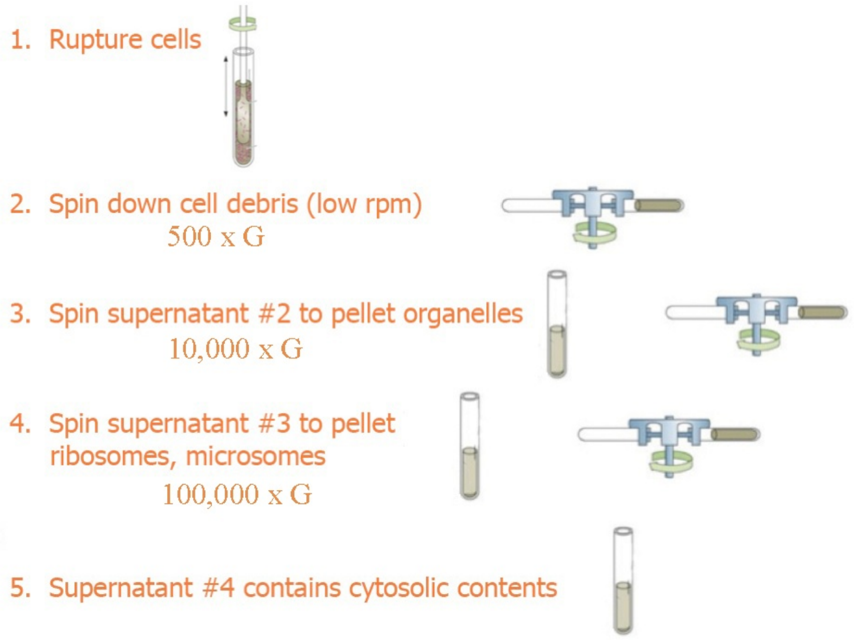
Another means of disrupting cells involves using a “cell bomb”. In this method, cells are placed under very high pressure (up to 25,000 psi). When the pressure is released, the rapid pressure change causes dissolved gases in cells to be released as bubbles which, in turn, break open the cells.

Cryopulverization is often employed for samples having a tough extracellular matrix, such as connective tissue or seeds. In this technique, tissues are flash-frozen using liquid nitrogen and then ground to a fine powder before extraction of cell contents with a buffer.

Whatever method is employed, the crude lysates obtained contain all of the molecules in the cell, and thus, must be further processed to separate the molecules into smaller subsets, or fractions.

Fractionation

Fractionation of samples typically starts with centrifugation. Using a centrifuge, one can remove cell debris, and fractionate organelles, and cytoplasm. For example, nuclei, being relatively large, can be spun down at fairly low speeds. Once nuclei have been sedimented, the remaining solution, or supernatant, can be centrifuged at higher speeds to obtain the smaller organelles, like mitochondria. Each of these fractions will contain a subset of the


1. Rupture cells
2. Spin down cell debris (low rpm)
500 x G
3. Spin supernatant #2 to pellet organelles
10,000 x G
4. Spin supernatant #3 to pellet ribosomes, microsomes
100,000 x G
5. Supernatant #4 contains cytosolic contents

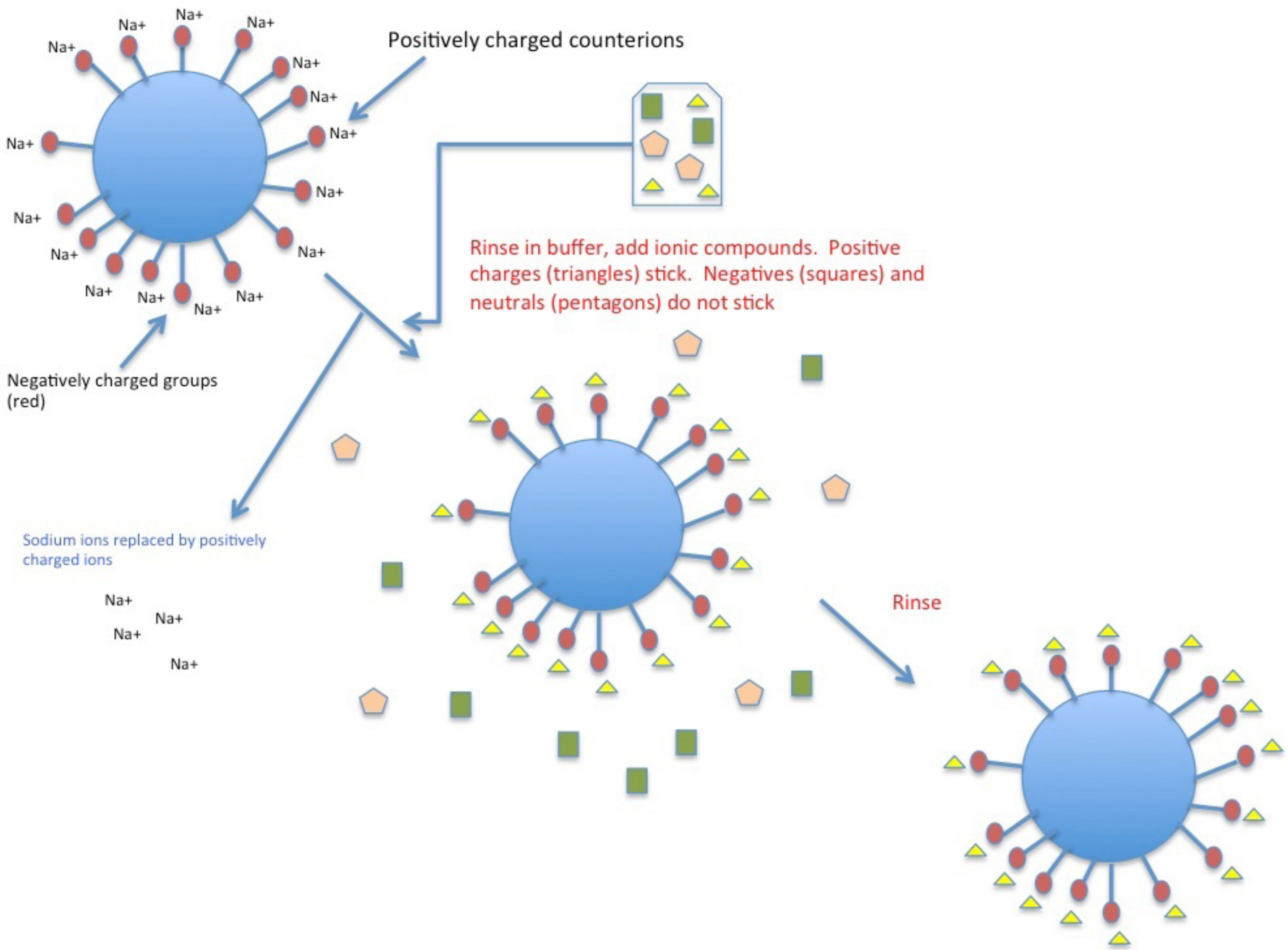
Fractionation by Centrifugation

molecules in the cell. Although every subset contains fewer molecules than does the crude lysate, there are still many hundreds of molecules in each. Separating the molecule of interest from the others is where chromatography comes into play. We will consider several separation techniques.

Many chromatographic techniques are performed in "columns." These are tubes containing the material (called the "support") used to perform the separation . Supports are designed to exploit the chemical, or size, differences of the many molecules in a mixture. Columns are "packed" (filled) with the support and a buffer or solvent carries the mixture of compounds to be separated through the support. Molecules in the sample interact differentially with the support and consequently, will travel through it with different speeds.

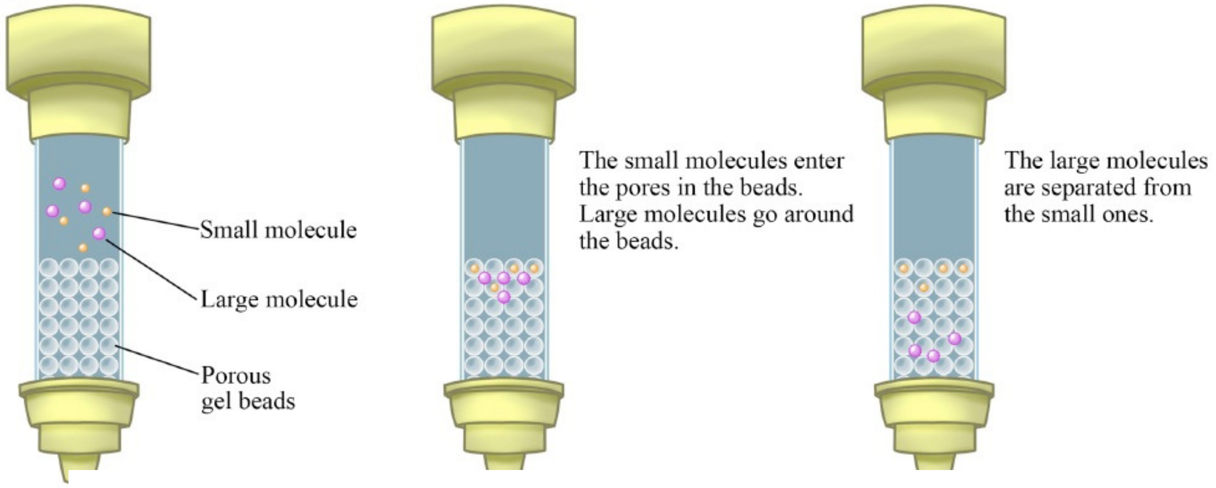
Ion Exchange Chromatography

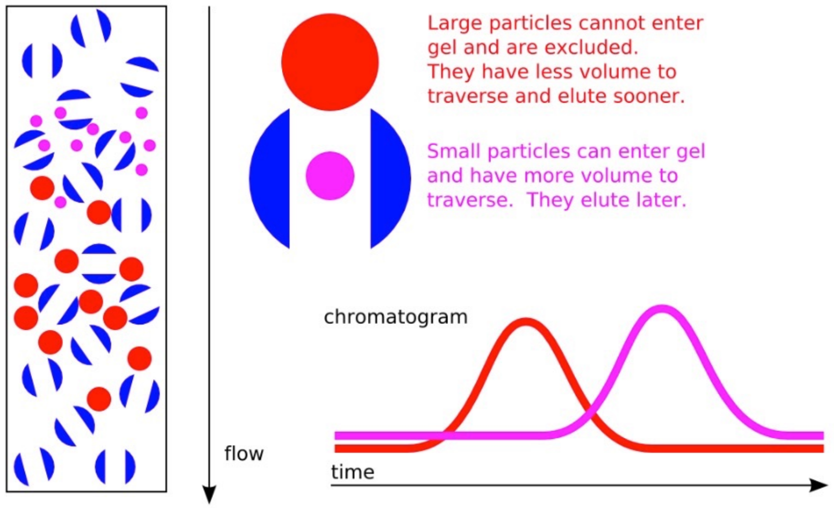
In ion exchange chromatography, the support consists of tiny beads to which are attached chemicals possessing a charge. Each charged molecule has a counter-ion. The figure shows the beads (blue) with negatively charged groups (red) attached. In this example, the counter-ion is sodium, which is positively charged. The negatively charged


Na+
Positively charged counterions
Negatively charged groups (red)
Rinse in buffer, add ionic compounds. Positive charges (triangles) stick. Negatives (squares) and neutrals (pentagons) do not stick
Sodium ions replaced by positively charged ions
Rinse

Cation Exchange Chromatography

groups are unable to leave the beads, due to their covalent attachment, but the counter-ions can be “exchanged” for molecules of the same charge. Thus, a cation exchange column will have positively charged counter-ions and positively charged compounds present in a mixture passed through the column will exchange with the counter-ions and “stick” to the negatively charged groups on the beads. Molecules in the sample that are neutral or negatively charged will pass quickly through the column. On the other hand, in anion exchange chromatography, the chemical groups attached to the beads are positively charged and the counter-ions are negatively charged. Molecules in the sample that are negatively charged will “stick” and other molecules will pass through quickly. To remove the molecules “stuck” to a column, one simply needs to add a high concentration of the appropriate counter-ions to displace and release them. This method allows the recovery of all components of the mixture that share the same charge.


Small molecule
Large molecule
Porous gel beads
The small molecules enter the pores in the beads. Large molecules go around the beads.
The large molecules are separated from the small ones.


Large particles cannot enter gel and are excluded. They have less volume to traverse and elute sooner.
Small particles can enter gel and have more volume to traverse. They elute later.
chromatogram
flow
time

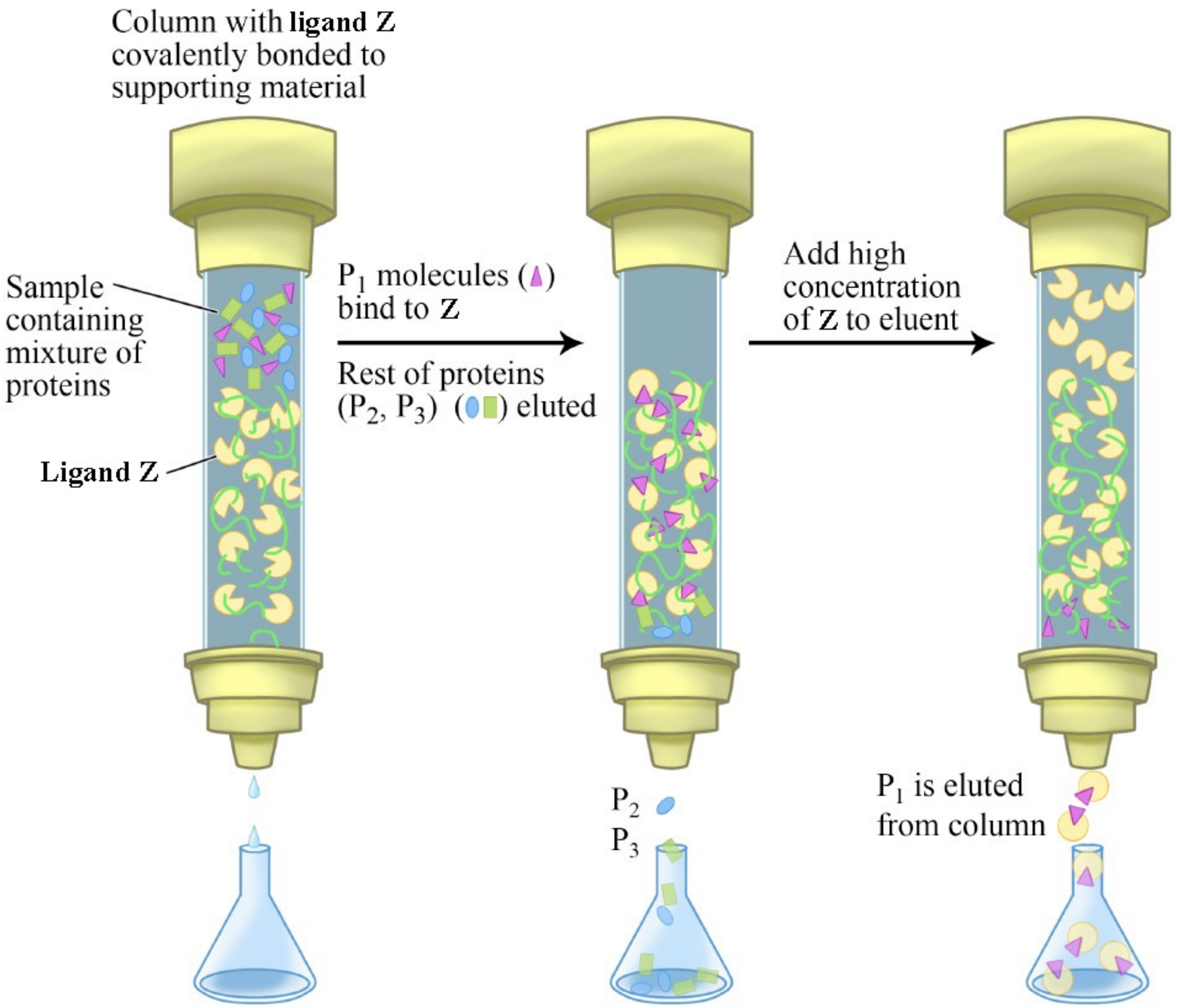
Gel Exclusion Chromatography

https://en.wikipedia.org/wiki File:SizeExChrom.png

Gel Exclusion Chromatography

Gel exclusion chromatography (also called molecular exclusion chromatography, size exclusion chromatography, or gel filtration chromatography) is a low resolution isolation method that employs a cool “trick.” This involves the use of beads that have tiny “tunnels” in them that each have a precise size. The size is referred to as an “exclusion limit,” which means that molecules above a certain molecular weight will not fit into the tunnels. Molecules with sizes larger than the exclusion limit do not enter the tunnels and pass through the column relatively quickly by making their way between the beads. Smaller molecules, which can enter the tunnels, do so, and thus, have a longer path

Click HERE and HERE, for Kevin’s Techniques lectures on YouTube that they take in passing through the column. Because of this, molecules larger than the exclusion limit will leave the column earlier, while those that pass through the beads will elute from the column later. This method allows separation of molecules by their size.


Column with **ligand Z** covalently bonded to supporting material
Sample containing mixture of proteins
Ligand Z
P_1 molecules (▲) bind to Z
Rest of proteins (P_2, P_3) (● ■) eluted
Add high concentration of Z to eluent
P_2
P_3
P_1 is eluted from column

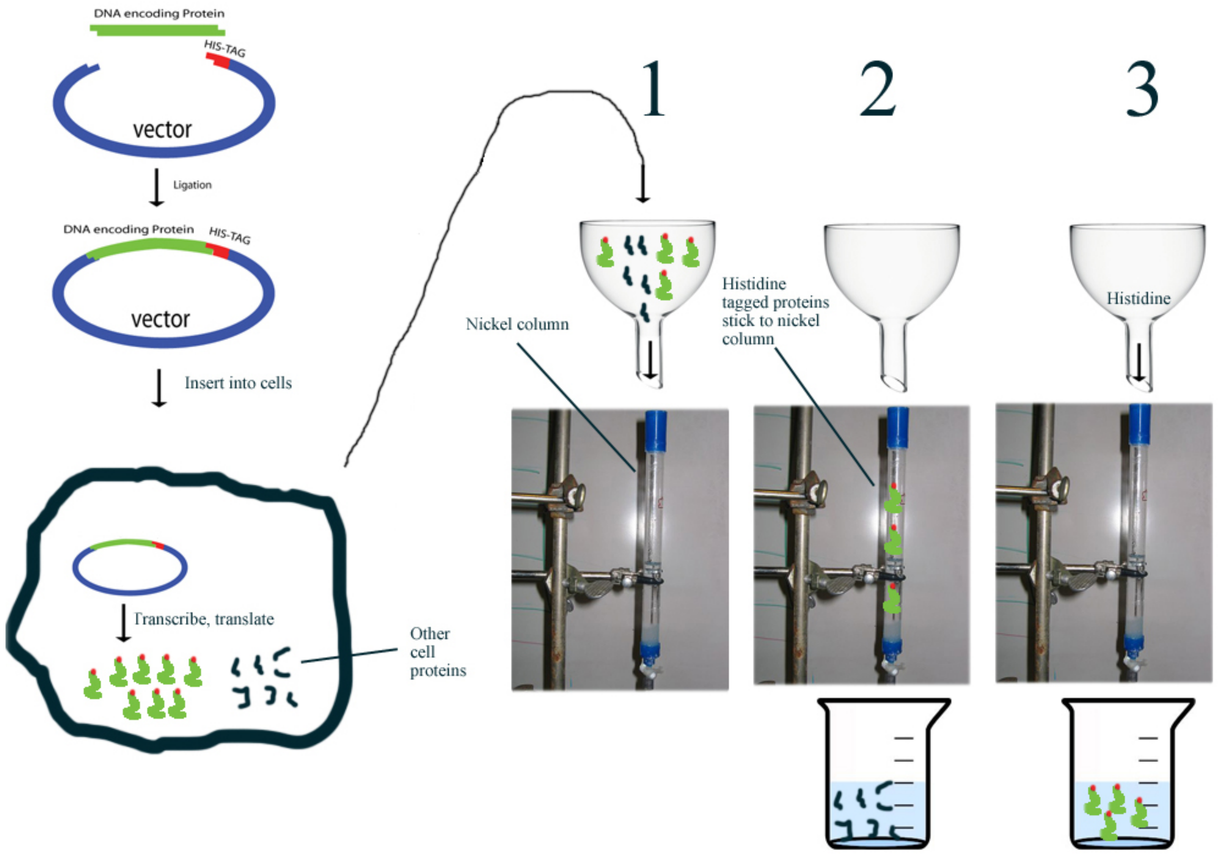
Affinity Chromatography

Affinity Chromatography

Affinity chromatography is a very powerful technique that exploits the binding affinities of target molecules (typically proteins) for substances covalently linked to beads. For example, if one wanted to separate all of the proteins in a sample that bound to ATP from proteins that do not bind ATP, one could covalently link ATP to support beads and then pass the sample through column. All proteins that bind ATP will “stick” to the column, whereas those that do not bind ATP will pass quickly through it. The proteins adhering to the column may then released from the column by adding ATP.

High Performance Liquid Chromatography (HPLC)

HPLC (also sometimes called High Pressure Liquid Chromatography) is a powerful tool for separating smaller molecules based on their differential polarities. It employs columns with supports made of very tiny beads that are so tightly packed that flow of solvents/buffers through the columns requires the application of high pressures (hence the name). The supports used can be polar (normal phase separation) or non-polar (reverse phase separation). In normal phase separations, non-polar molecules elute first followed by the more polar compounds. This order is


DNA encoding Protein
HIS-TAG
vector
Ligation
DNA encoding Protein
HIS-TAG
vector
Insert into cells
Transcribe, translate
Other cell proteins
1
2
3
Nickel column
Histidine tagged proteins stick to nickel column
Histidine

Histidine Tagging

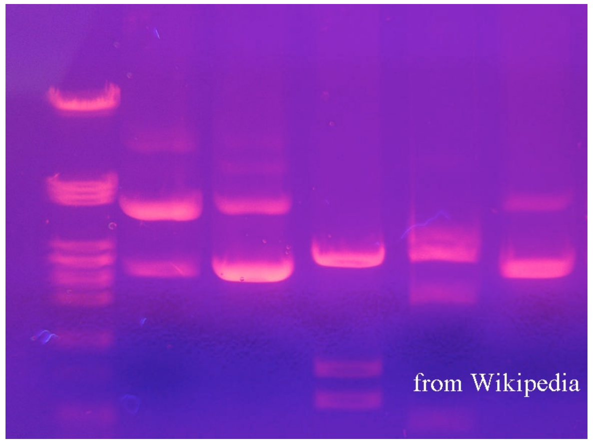
switched in reverse phase chromatography. Of the two, reverse phase is much more commonly employed to due more reproducible chromatographic profiles (separations) that it typically produces.

Histidine Tagging

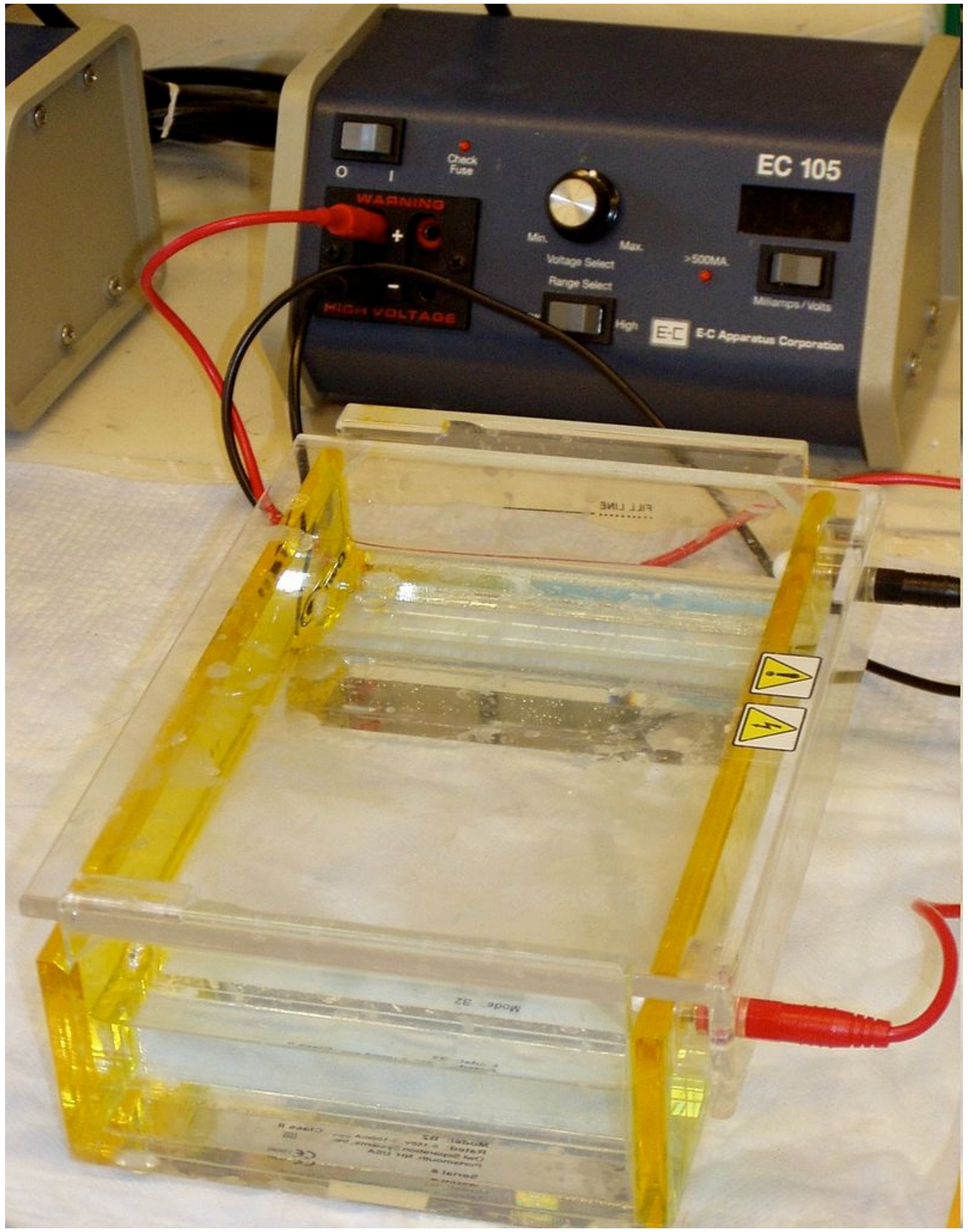
Histidine tagging is a powerful tool for isolating a recombinant protein from a cell lysate. It relies on using recombinant DNA techniques to add codons specifying a series of histidines (usually six) to the coding sequence for a protein. The protein produced when this gene is expressed has a run of histidine residues fused at either the carboxyl or amino terminus to the amino acids in the remainder of the protein. The histidine side chains of this “tag” have an affinity for nickel or cobalt ions, making separation of histidine-tagged proteins from a cell lysate is relatively easy. Simply passing the sample through a column that has immobilized nickel or cobalt ions allows the histidine-tagged proteins to “stick,” while the remaining cell proteins all pass quickly through. The histidine-tagged proteins are then eluted by addition of imidazole (which is chemically identical to the histidine side chain) to the column. Histidine tags can be cleaved off using endopeptidases.

Electrophoresis

DNA molecules are long and loaded with negative charges, thanks to their phosphate backbones. Electrophoretic methods separate large molecules, such as DNA, RNA, and proteins based on their charge and size. For DNA and RNA, the charge of the nucleic acid is proportional to its size (length). For proteins, which do not have a


from Wikipedia

DNA Fragments Separated by Agarose Gel Electrophoresis



Agarose Gel Electrophoresis

uniform charge, a clever trick is employed to make them mimic nucleic acids.

Agarose Gel Electrophoresis

Agarose gel electrophoresis is a method for separating nucleic acids. It is worth noting that nucleic acids are the largest molecules found in cells, in some cases by orders of magnitude. Agarose provides a matrix which encases a buffer. The matrix provides openings for macromolecules to move through and the largest macromolecules have the most difficult time navigating, whereas the smallest macromolecules slip through it the easiest. Unlike column chromatography, electrophoresis uses an electric current as a force to drive the molecules through the matrix. Since the size to charge ratios for DNA and RNA are constant for all sizes of these nucleic acids, the size per force is also constant (since force is directly proportional to charge), so the molecules simply sort on the basis of their size - the smallest move fastest and the largest move slowest. Visualization of the DNA fragments in the gel is

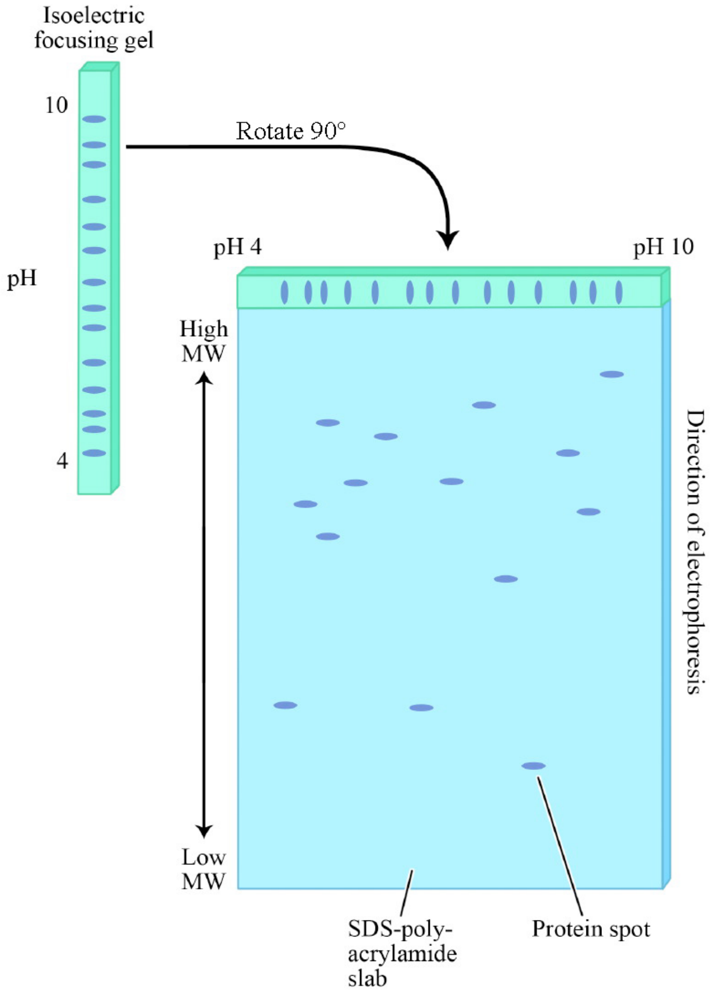
from Wikipedia

SDS-PAGE Separation of Proteins

made possible by addition of a dye, such as ethidium bromide that fluoresces under ultraviolet light.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Like DNA and RNA, proteins are large macromolecules. Proteins, however, vary tremendously in their charge. Whereas double-stranded DNA is rod-shaped, most proteins are globular (folded up). Further, proteins are considerably smaller than nucleic acids, so the openings of the matrix of the agarose gel are simply too large to effectively provide separation. Consequently, unlike nucleic acids, proteins cannot be effectively separated by electrophoresis on agarose gels. To separate proteins by electrophoresis, one must make several modifications. First, a matrix made by polymerizing and crosslinking acrylamide units is employed. One can adjust the pore size of the matrix readily by changing the percentage of acrylamide in the gel. Higher percentages of acrylamide create smaller pores and are more effective in separating smaller molecules, whereas lower percentages of acrylamide reverse that. Second, proteins must be physically altered to "present" themselves to the matrix like the negatively charged rods of DNA. This is accomplished by treating the proteins with the detergent called SDS (sodium dodecyl sulfate). SDS denatures the proteins so they assume a rod-like shape and the SDS molecules coat the proteins such that the exterior surface is loaded with negative charges proportional to the mass, just like the backbone of DNA. Third, a "stacking gel" may be employed at the top of the gel to provide a way of compressing the samples into a tight band before they enter the main polyacrylamide gel (called the resolving gel). Just as DNA fragments get sorted on the basis of size (largest move slowest and smallest move fastest), the proteins migrate through the gel matrix at rates inversely related to their size. Upon completion of the electrophoresis, there are several means of staining to visualize the proteins on the gel. They include reagents, such as Coomassie Brilliant Blue or silver nitrate (the latter is much more sensitive than Coomassie Blue staining and can be used when there are very small quantities of protein).



2D Gel Electrophoresis

Click HERE and HERE, for Kevin's Techniques lectures on YouTube

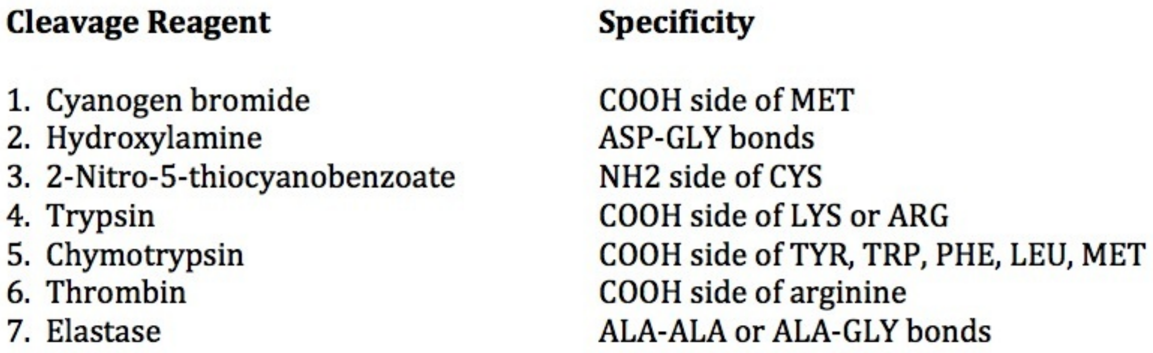
Isoelectric Focusing

Proteins vary considerably in their charges and, consequently, in their pI values (pH at which their charge is zero). Separating proteins by isoelectric focusing requires establishment of a pH gradient in an acrylamide gel matrix. The matrix's pores are adjusted to be large to reduce the effect of sieving based on size. Molecules to be focused are applied to the gel with the pH gradient and an electric current is passed through it. Positively charged molecules, for example, move towards the negative electrode, but since they are traveling through a pH gradient, as they pass through it, they reach a region where their charge is zero and, at that point, they stop moving. They are at that point attracted to neither the positive nor the negative electrode and are thus "focused" at their pI. By using isoelectric focusing, it is possible to separate proteins whose pI values differ by as little as 0.01 units.

2D Gel Electrophoresis

Both SDS-PAGE and isoelectric focusing are powerful techniques, but a clever combination of the two is a powerful tool of proteomics - the science of studying all of the proteins of a cell/tissue simultaneously. In 2D gel electrophoresis, an extract containing the proteins is first prepared. One might, for example, be studying the proteins of liver tissue. The liver cells are lysed and all of the proteins are collected into a sample. Next, the sample is subjected to isoelectric focusing as described earlier, to separate the proteins by their pI values. Next, as shown on the previous page, the isoelectric gel containing the separated proteins is rotated through 90º and placed on top of a regular polyacrylamide gel for SDS-PAGE analysis (to separate them based on size). The proteins in the isoelectric gel matrix are electrophoresed into the polyacrylamide gel and separation on the basis of size is performed. The product of this analysis is a 2D gel, in which proteins are sorted by both mass and charge.

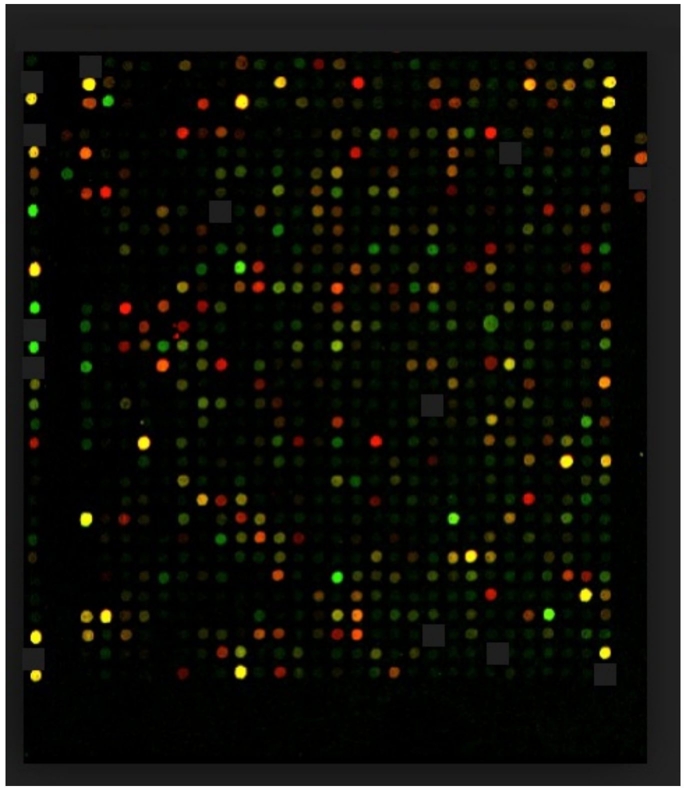
The power of 2D gel electrophoresis is that virtually every protein in a cell can be separated and appear on the gel as a distinct spot. In the figure, spots in the upper left correspond to large positively charged proteins, whereas those in the lower right are small negatively charged ones. It is possible using high-throughput mass spectrometry analysis to identify every spot on a 2D gel. This is particularly powerful when one compares protein profiles between different tissues or between the samples of the same tissue treated or untreated with a particular drug.



Cleavage Reagent	Specificity
1. Cyanogen bromide	COOH side of MET
2. Hydroxylamine	ASP-GLY bonds
3. 2-Nitro-5-thiocyanobenzoate	NH2 side of CYS
4. Trypsin	COOH side of LYS or ARG
5. Chymotrypsin	COOH side of TYR, TRP, PHE, LEU, MET
6. Thrombin	COOH side of arginine
7. Elastase	ALA-ALA or ALA-GLY bonds

Protein Cleaving Agents

Comparison of a 2D separation of a non-cancerous tissue with a cancerous tissue of the same type provides a quick identification of proteins whose level of expression differs between them. Information such as this might be useful in designing treatments or in determining the mechanisms by which the cancer arose.



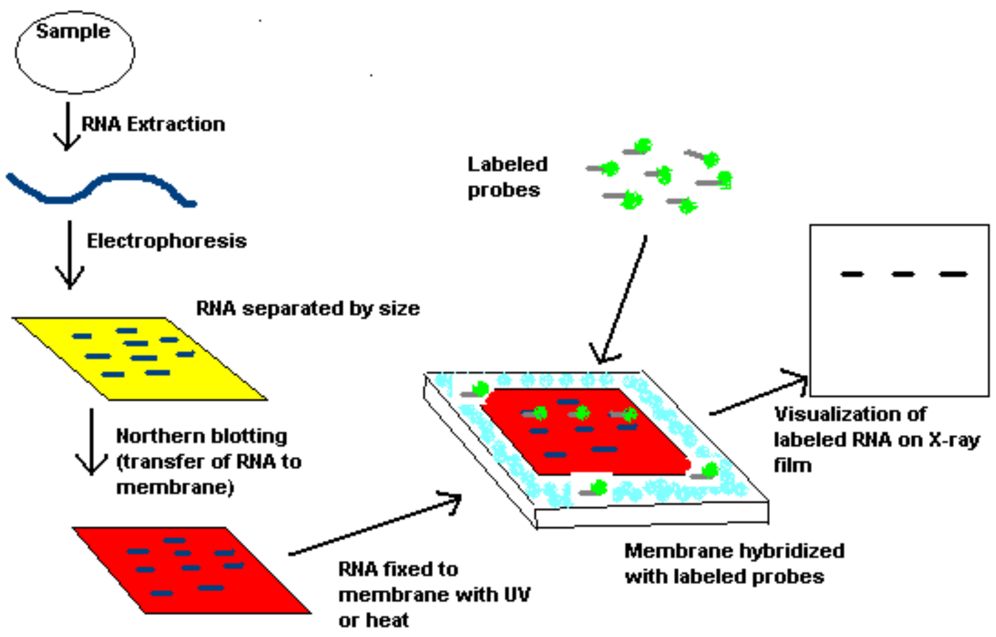
Microarray

Protein Cleavage

Working with intact proteins in analytical techniques, such as mass spectrometry, can be problematic. Consequently, it is often desirable to break a large polypeptide down into smaller, more manageable pieces. There are two primary approaches to accomplishing this - use of chemical reagents or use of proteolytic enzymes. The table on the previous page shows the cutting specificities of various cleavage agents.

Microarrays

2D gels are one way of surveying a broad spectrum of molecules simultaneously. Other approaches to doing the same thing involve what are called microarrays. DNA microarrays, for example, can be used to determine all of the genes that are being expressed in a given tissue, simultaneously. Microarrays employ a grid (or array) made of rows and columns on a glass slide, with each box of the grid containing many copies of a specific molecule, say a single-stranded DNA molecule corresponding to the sequence of a single unique gene. As an example, consider scanning the human genome for all of the known mRNA sequences and then synthesizing single stranded DNAs complementary to each mRNA. Each complementary DNA sequence would have its own spot on the matrix. The position of each unique gene sequence on the grid is known and the entire grid would represent all possible genes that are expressed. Then for a simple gene expression analysis, one could take a tissue (say liver) and extract the mRNAs from it. These mRNAs represent all the genes that are being expressed in the liver at the time the extract was made. The mRNAs can easily be tagged with a colored dye (say blue). The mixture of tagged mRNAs is then added to the array and base-pairing conditions are created to allow complementary sequences to find each other. When the process is complete, each liver mRNA should have bound to its corresponding gene on the array, creating a blue spot in that box on the grid. Since it is known which genes are in which box, a blue spot in a box indicates that the gene in that box was expressed in the liver. The presence and abundance of each mRNA may then readily determined by measuring the amount of blue dye at each box of the grid. A more powerful analysis could be performed with two sets of mRNAs, each with a different colored tag (say blue and yellow). One set of mRNAs could come from the liver of a vegetarian (tagged blue) and the other from a meat eater (tagged yellow), for example. The mRNAs are mixed and then added to the array and complementary sequences are once again allowed to form duplexes. After unhybridized mRNAs are washed away, the plate is analyzed. Blue spots in grid boxes correspond to mRNAs present in the vegetarian liver, but not in that of the meat eater. Green spots (blue plus yellow) would correspond to mRNAs present in equal abundance in the two livers. The intensity of each spot would also give information about the relative amounts of each mRNA in the tissues. Similar analyses could be done, using cDNAs instead of mRNA. Peptide microarrays have peptides bonded to the glass slide instead of DNA and can be used to study the binding of proteins or other molecules to the peptides.


Sample
RNA Extraction
Electrophoresis
RNA separated by size
Labeled probes
Northern blotting (transfer of RNA to membrane)
Visualization of labeled RNA on X-ray film
RNA fixed to membrane with UV or heat
Membrane hybridized with labeled probes

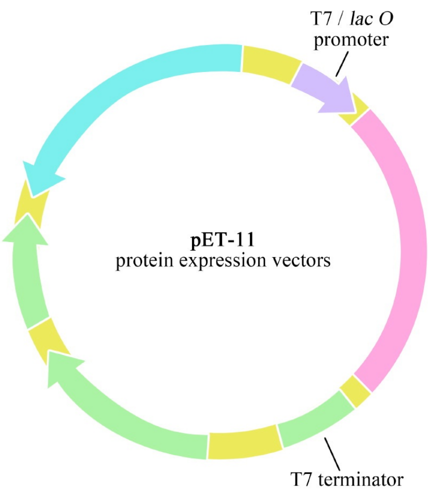
Northern Blotting Procedure

Blotting

Blotting provides a means of identifying specific molecules out of a mixture. It employs three main steps. First, the mixture of molecules is separated by gel electrophoresis. The mixture could be DNA (Southern Blot), RNA (Nothern Blot), or protein (Western Blot) and the gel could be agarose (for DNA/RNA) or polyacrylamide (for protein). Second, after the gel run is complete, the proteins or nucleic acids in the gel are transferred out of the gel onto a membrane/paper that physically binds to the molecules. This "blot", as it is called, has an imprint of the bands of nucleic acid or protein that were in the gel (see figure at left). The transfer can be accomplished by diffusion or by using an electrical current to move the molecules from the gel onto the membrane. The membrane may be treated to covalently link the bands to the surface of the blot. Last, a visualizing agent specific for the molecule of interest in the mixture is added to the membrane. For DNA/RNA, that might be a complementary nucleic acid sequence that is labeled in some fashion (radioactivity or dye). For a protein, it would typically involve an antibody that specifically binds to the protein of interest. The bound antibody can then be targeted by another antibody specific for the first antibody. The secondary antibody is usually linked to an enzyme which, in the presence of the right reagent, catalyzes a reaction that produces a signal (color or light) indicating where the antibody is bound. If the molecule of interest is in the original mixture, it will “light” up and reveal itself.

Making Recombinant DNAs

Molecular biologists often create recombinant DNAs by joining together DNA fragments from different sources. One reason for making recombinant DNA molecules is to enable the production of a specific protein that is of interest. For example, it is possible to engineer a recombinant DNA molecule containing the gene for human growth hormone and introduce it into an organism like a bacterium or yeast, which could make massive quantities of the human growth hormone protein very cheaply. To do this, one needs to set up the proper conditions for the protein to be made in the target cells. For bacteria, this typically involves the use of plasmids. Plasmids are circular, autonomously replicating DNAs found commonly in bacterial cells. Plasmids used in recombinant DNA methods 1) replicate in high numbers in the host cell; 2) carry markers that allow researchers to identify cells carrying them (antibiotic resistance, for example) and 3) contain sequences (such as a promoter and Shine Dalgarno sequence) necessary for expression of the desired protein in the target cell. A plasmid that has all of these features is referred to as an expression vector (see an example in the figure at left). Plasmids may be extracted from the host, and any gene of interest may be inserted into them, before returning them to the host cell. Making such recombinant plasmids is a relatively simple process. It involves 1) cutting the gene of interest with a restriction enzyme (endonucleases which cut at specific DNA sequences); 2) cutting the expression plasmid DNA with restriction enzyme, to generate ends that are compatible with the ends of the gene of interest; 3) joining the gene of interest to the plasmid DNA using DNA ligase; 4) introducing the recombinant plasmit into a bacterial cell; and 5) growing cells that contain the plasmid. The bacterial cells bearing the recombinant plasmid may then be induced to express the inserted gene and produce large quantities of the protein encoded by it.



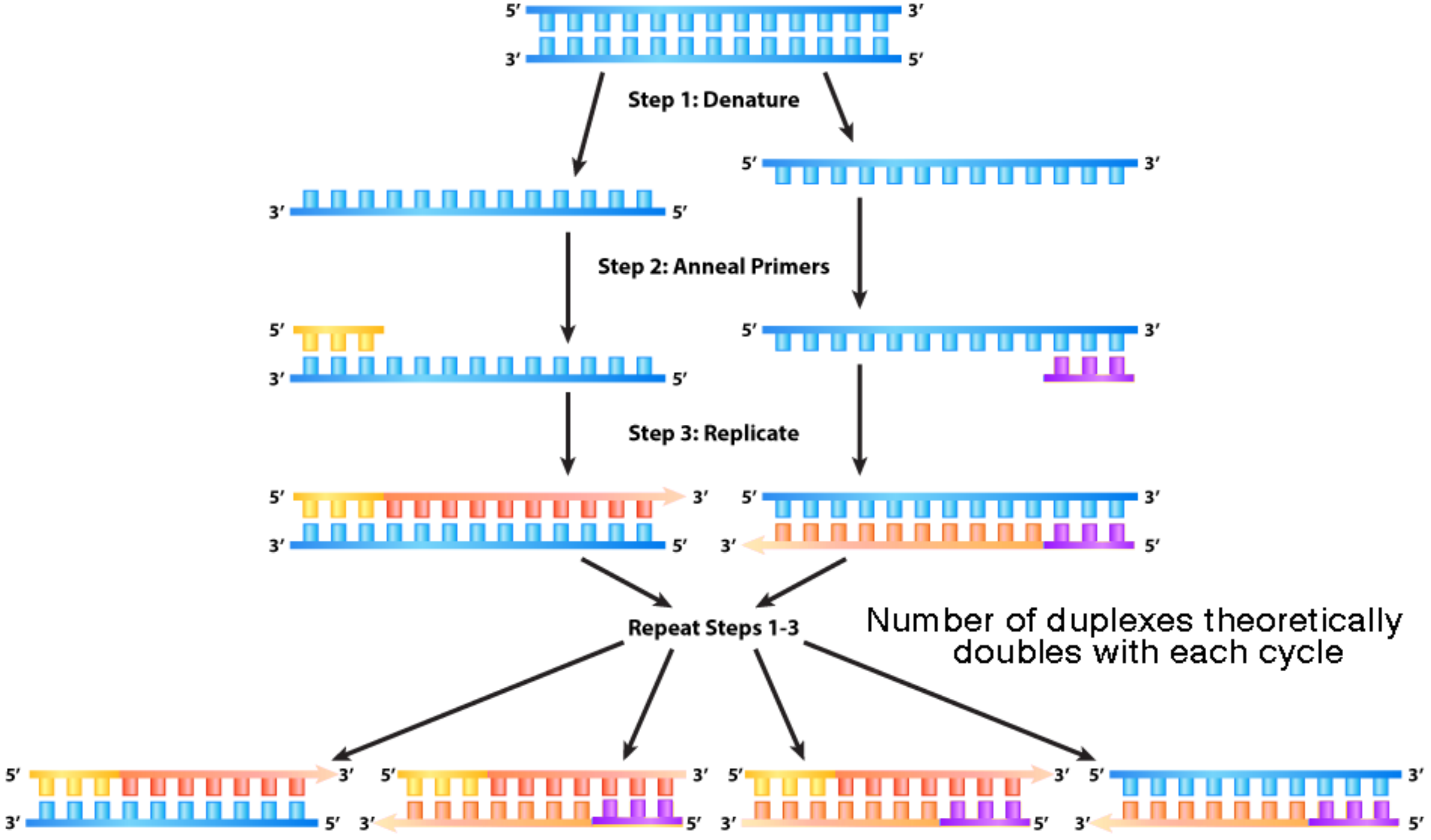
An Expression Vector

Polymerase Chain Reaction (PCR)

PCR allows one to use the power of DNA replication to obtain large amounts of a specific DNA in a short time. As everyone

Click HERE and HERE, for Kevin's Techniques lectures on YouTube

knows, cell division results in doubling the number of cells with each round of division. Each time cells divide, DNA must be replicated, as well, so the amount of DNA is doubling as the cells are doubling. Kary Mullis recognized this fact and came up with the technique of PCR, which mimics DNA replication. In contrast to cellular DNA replication, which amplifies all of a cell's DNA during a replication cycle, PCR is used to replicate only a specific segment of DNA. This segment of DNA, known as the target sequence, is replicated repeatedly, to obtain millions of copies of the target. Just as in DNA replication, PCR requires a template DNA, 4 dNTPs, primers to initiate DNA synthesis on each strand, and a DNA polymerase to synthesize the new DNA copies. In PCR, the primers bind to sequences flanking the target region that is to be amplified, and are present in large excess over the template. The DNA polymerase used is chosen to be heat stable, for reasons that will be clear shortly. The first step of each PCR cycle involves separating the strands of the template DNA so that it can be replicated. This is accomplished by heating the DNA to near boiling temperatures. In the next step of the cycle, the solution is cooled to a temperature that favors complementary DNA sequences finding each other. Since the primers are present in great excess over the template, they can readily find and base-pair with the complementary sequences in the template on either side of the target sequence. In the third step in the cycle, the DNA polymerase (which has not been denatured during the heat

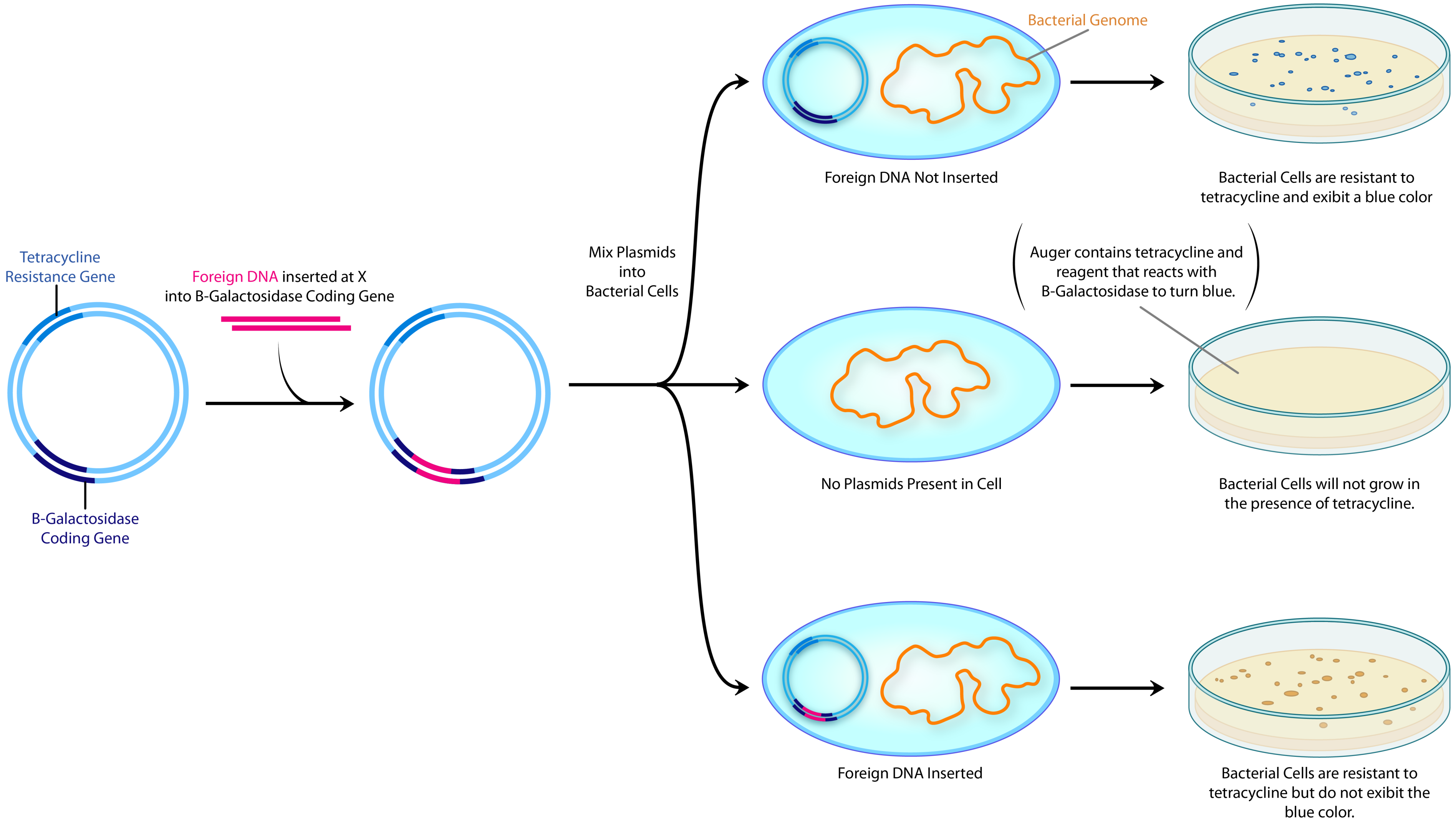


PCR

treatment because it is thermostable) extends the primer on each strand, making copies of both DNA strands and doubling the amount of the target sequence. The cycle is then repeated, usually about 30 times. At the end of the process, there is a theoretical yield of 2^{30} more of the target DNA than there was in the beginning.

Lac Z Blue-White Screening

A powerful tool for biotechnologists is the *lac Z* gene. You may recall from an earlier section on the control of gene expression, that *lac Z* is part of the *lac* operon of *E. coli* and encodes the enzyme β galactosidase. This enzyme catalyzes the hydrolysis of lactose into glucose and galactose, allowing the bacteria to use lactose as an energy source. β galactosidase can also break down an artificial substrate called X-gal to produce a compound that is blue in color. X-gal can thus be used to test for the presence of active β galactosidase. With this background, we can now look at how the *lac Z* gene can be of help to molecular biologists when they create recombinant plasmids. In the example described earlier, the gene for human growth hormone (hGH) was inserted into a plasmid. As we noted, the plasmid, as well as the hGH gene are cut with restriction


Tetracycline
Resistance Gene
B-Galactosidase
Coding Gene
Foreign DNA inserted at X
into B-Galactosidase Coding Gene
Mix Plasmids
into
Bacterial Cells
Bacterial Genome
Foreign DNA Not Inserted
Bacterial Cells are resistant to
tetracycline and exibit a blue color
Auger contains tetracycline and
reagent that reacts with
B-Galactosidase to turn blue.
No Plasmids Present in Cell
Bacterial Cells will not grow in
the presence of tetracycline.
Foreign DNA Inserted
Bacterial Cells are resistant to
tetracycline but do not exibit the
blue color.

Blue-White Screening Strategy

endonucleases to create compatible DNA ends that can be ligated. While the ends of the hGH gene are, indeed, capable of being ligated to the ends of the plasmid, the two ends of the plasmid could also readily rejoin. In fact, given that the two ends of the plasmid are are on the same molecule, the chances of their finding each other are much higher than of a plasmid end finding an hGH gene. This would mean that many of the ligated molecules would not be recombinants, but simply recircularized plasmids. Five percent of the plasmids having inserts of the hGH gene would be very good. That would mean that 95% of the bacterial colonies arising from transformation would contain the original plasmid rather than the recombinant. To make the process of screening for the relatively rare recombinants simpler, plasmids have been engineered that carry the *lac Z* gene, modified to contain, with the coding sequence, restriction enzyme recognition sites. If one of these sites is used to cut open the plasmid and a gene of interest is inserted, this disrupts the *lac Z* gene. If the plasmid simply recircularizes, the *lac Z* gene will be intact. To find which bacterial colonies carry the recombinant plasmids, X-Gal is provided in the plates. Bacterial colonies containing plasmids with the *lac z* sequence disrupted by an inserted gene will not produce functional β galactosidase. The X-Gal will not be broken down and there will be no blue color. By contrast, bacterial cells with recircularized plasmids having no inserted hGH gene will make functional β galactosidase, so in the presence of X-Gal and IPTG these colonies will produce a blue color. This is summarized in the figure on the previous page.

Reverse Transcription

According to the central dogma, DNA codes for mRNA, which codes for protein. An exception to this rule is seen with the retroviruses, RNA-encoded viruses that have a phase in their replication cycle during which their genomic RNA is copied into DNA by a virally-encoded enzyme known as reverse transcriptase. The ability to convert RNA to DNA can be useful in the laboratory. For example, the power of PCR can be brought to RNA by converting RNAs of interest to DNA and then amplifying them by PCR. With reverse transcriptase, this is readily accomplished. First, one creates a DNA oligonucleotide to serve as a primer for reverse transcriptase to use on a target RNA. The primer must, of course, be complementary to a segment (near the 3’ end) of the RNA to be amplified. The RNA template, reverse transcriptase, the primer, and four dNTPs are mixed. With one round of replication, the RNA is converted to a single strand of DNA, which can be separated from the RNA either by heating or by the use of an RNase to digest the RNA. The product of this process is called a complementary DNA (cDNA).

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Chapter 10

Putting It All Together

With this chapter, we tie up a bunch of loose ends and ponder what lies in the future of biochemistry

Putting Everything Together

> *"Organic chemistry is the study of carbon compounds. Biochemistry is the study of carbon compounds that crawl"*
>
> *-Mike Adam.*

Looking Back

The bounds of biochemistry have expanded enormously since its inception. Wöhler's demonstration, in 1828, that urea could be synthesized outside of a living cell, showed that there was no "vital force" that distinguished the chemistry of life from that of the non-living world. Chemistry is chemistry, but the term "biochemistry" was coined in 1903 by Carl Neuberg to describe the special subset of chemical reactions that happen in living cells. This specialness derives not from any exceptions to the laws of physics and chemistry, but from the way in which the chemical reactions in cells are organized and regulated, and also from the complexity and size of biological molecules.

Faced with far greater complexity than in the inorganic world, the traditional strategy of biochemists has been "divide and conquer." In this approach, individual enzymes and other biological molecules are purified from cells so that their properties can be studied in isolation. The underlying logic of this method, sometimes described as reductionist, is that we can learn about the whole by studying its individual parts. This painstaking approach, used through most of the twentieth century, teased out chemical reactions and molecular interactions that occur within cells, one by one, gradually revealing to scientists much of what we know in biochemistry today.

Biochem is Beautiful

To the tune of “*Everything is Beautiful*”

Students study molecules with
All of the structures they possess
Proteins, fats and DNAs
There must be a million ways
To evaluate our knowledge for the test

Biochem is beautiful
Our professor says
From the sugar in our cells
To actions of HDLs

And molecules are dutiful
In every way
Substrates for the enzymes are
Converted e-ver-y day

There is no enzyme
That can lower Delta G
They just work all the time
On transition energy

Catalysis provides to cells
Metabolic jump-startin
They all capitalize
By giving rise
To reactions ‘tween the carbons

Biochem is beautiful
Saying it with zest
Would be so much easier
If I could just ace the test

Biochem is beautiful
Saying it with zest
Would be so much easier
If I could just ace the test

Recorded by David Simmons
Lyrics by Kevin Ahern

As increasing numbers of biochemical reactions were worked out, biochemists began to see that they were connected together in chains of reactions that we now refer to as metabolic pathways. These metabolic pathways turned out to be remarkably similar between cells across all kingdoms of life. Though there are a few pathways that are unique to certain organisms, many more are the same, or very similar, in organisms as different as bacteria and humans.

It also became clear that metabolic pathways interacted with each other *via* common intermediates or by regulation of one pathway by molecule(s) created by other pathway(s). The similarity of the chemical reactions in all living cells was shown to extend to the common energy currency, ATP, that cells use to power their chemical reactions, as well as the mechanism by which cells make the ATP.

Metabolic pathways trace the transformation of molecules in a cell and represent the work of enzymes, which are proteins. The discovery of the structure of DNA led to understanding of how information in genes was used to direct the synthesis of these proteins. The protein-DNA interactions that determine which genes are copied into RNA at any given time were uncovered and helped explain how cells with the same DNA came to express different proteins. The genetic code, as well as the mechanisms of transcription, translation and regulation of gene expression also turned out to be remarkably similar in cells of all kinds, leading Nobel laureate Jacob Monod to joke that what was true for *E.coli* was also true for *E.lephant*.

The “one component at a time” approach also helped biochemists understand how cells sense changes in their environment and respond to them. The ability to sense conditions outside the environs of cells extends through all groups of organisms. Even the simplest single-celled organism can follow nutrient gradients to move itself closer to food. Cells in multicellular organisms can detect chemical cues in the blood (nutrients, hormones) or impulses from nerve cells and alter their actions. These cues may trigger changes in metabolism, decisions to divide, die, or become senescent, or the performance of specialized functions (e.g., muscle contraction or enzyme secretion). Thus cells are constantly in a state of flux, adjusting their activities in response to signals from outside themselves as well as their own changing needs.

> *“We are only now beginning to acquire reliable material for welding together the sum total of all that is known into a whole “*
>
> *- Erwin Schrodinger, 1944*

The power of the “take things apart” analytical approach is evident from the astounding pace of discoveries in biochemistry and molecular biology. The first demonstration that an enzyme was a protein was made only in 1926, and it wasn’t till twenty years later that this was sufficient well established that the Nobel Prize was awarded in 1946 for this discovery. Since that time, the methods of biochemistry have uncovered all of the information that you can find in any standard biochemistry textbook, and more.

Thousands of enzymes and their substrates have been identified, and hundreds of metabolic pathways traced. The structure of hundreds of proteins is known down to the position of every atom. Following the elucidation of the structure of DNA in 1953, scientists have discovered a dizzying number of facts about how information is stored, used and inherited in cells. Cloned and transgenic animals and gene therapy were a reality in less than 50 years. And the discoveries still keep coming.

Looking Forward

But toward the end of the twentieth century, new methods began to change the face of biochemistry. The launching of the Human Genome Project and the development of faster and cheaper sequencing technologies provided biochemists with entire genome sequences, not only of humans, but of numerous other organisms. Huge databases were set up to deal with the volume of sequence information generated by the various genome projects. Computer programs cataloged and analyzed these sequences, making sense of the enormous quantities of data.

Thank God There's a Video

To the tune of "*Thank God I'm a Country Boy*"

Students sing text in RED

There's a bundle of things a student oughta know
And Ahern's talk isn't really very slow
Learnin' ain't easy / the lectures kinda blow
Thank God there's a video

Well we've gone through the cycles and their enzymes too
Studying the regulation everything is new
I gotta admit that I haven't got a clue
What am I gonna do?

So I got me a note card and bought me a Stryer
Got the enzymes down and the names he requires
I hope that I can muster up a little more desire
Thank God there's a video

Just got up to speed about the NAD
Protons moving through Complex Vee
Electrons dance in the cytochrome C
Gotta hear the MP$_3$

Fatty acid oxidation makes the acetyl-CoA
Inside the inner matrix of the mitochondri-ay
It's very complicated, I guess I gotta say
Thank God there's a video

So I got me a note card and bought me a Stryer
Got the enzymes down and the names he requires
I hope that I can muster up a little more desire
Thank God there's a video

Replication's kind of easy in a simple kind of way
Copyin' the bases in the plasmid DNAs
Gs goes with Cs and Ts go with As
Thanks to polymerase

And the DNA's a template for the RNA
Helices unwinding at T-A-T-A
Termination happens, then the enzyme goes away
Don't forget the poly-A

So I got me a note card and bought me a Stryer
Got the enzymes down and the names he requires
I think that I can muster up a little more desire
Thank God there's a video

Recorded by David Simmons
Lyrics by Kevin Ahern

Protein coding regions of genomes could be identified and translated “in silico” to deduce the amino acid sequence of the encoded polypeptides. Comparisons could be made between the gene sequences of different organisms. In parallel with the growth of sequence information, more and more protein structures were determined, by using X-ray crystallography and NMR spectroscopy. These structures, too, were deposited in databases to be accessible to all scientists.

> *“It is an old saying, abundantly justified, that where sciences meet there growth occurs. It is true moreover to say that in scientific borderlands not only are facts gathered that [are] often new in kind, but it is in these regions that wholly new concepts arise.”*
>
> *– Sir Frederick Gowland Hopkins*

The accumulation of vast amounts of sequence and structure information went hand in hand with new and ambitious goals for biochemistry. Modern biotechnology techniques have provided tools for studying biochemistry in entirely new ways. The old ways of dividing and conquering to study individual reactions are now being supplemented by approaches that permit researchers to study cellular biochemistry in its entirety.

These fields of research, which collectively are often referred to as the ‘-omics’ include genomics (study of all the DNA of a cell), proteomics (study of all the proteins of a cell), transcriptomics (study of all the transciption products of a cell), and metabolomics (study of all the metabolic reactions of a cell), among others. As an example, let us consider proteomics. The field of proteomics is concerned with all of the proteins of a cell. Since proteins are the ‘workhorses’ of cells, knowing which ones are being made at any given time provides us with an overview of everything that is happening in the cells under specific conditions.

How is such an analysis performed? First, one extracts all of the proteins from a given cell type (liver, for example). Next, the proteins are separated in a two-step gel method, where the first step resolves proteins based on their charge and the second separates them by mass. The product of this analysis is a single gel (called a 2-D gel) on which all of the proteins have been separated. In the left-right orientation, they differ in their original charge and in the up/down orientation, they differ in their size.

By using such a technique, as many as 6000 cellular proteins can be separated and visualized as spots on a single gel. Robotic techniques allow excision of individual spots and analysis on mass spectrometers to identify every protein present in the original extract.

Why is this useful? There are several ways in which this information can be illuminating. For example, by comparing the proteins in a normal liver cell with those in a cancerous liver cell, one can quickly determine if there are any proteins that are expressed or missing only in the cancer cells. These differences between normal and cancerous cells may provide clues to the mechanisms by which the cancer arose or suggest ways to treat the cancer. Or, the same sort of analysis could be done on cells to find out about the effects of a hormone or drug treatment. Comparison of the proteins found in untreated and treated cells would give a global view of the protein changes resulting from the treatment.

> *“Almost all aspects of life are engineered at the molecular level, and without understanding molecules we can only have a very sketchy understanding of life itself.”*
>
> *— Francis Crick*

Similar analyses can be performed on the mRNA of cells, employing devices called microarrays. In this case, all the RNAs that are being made at the time that the cell extract is made can be identified by the signals generated when the RNAs hybridize with oligonucleotides complementary to their sequence, that are immobilized in ordered arrays on the surface of a plate. The position and strength of these signals indicates which RNAs are made and in what amounts.

The techniques of proteomics and transcriptomics, together with other “global view” approaches of molecules like lipids, carbohydrates, etc., are allowing biochemists to have, for the first time, a “big picture” view of the activities of cells. While these techniques have already provided valuable new insights, they are still incomplete, as a description of what goes on in cells. This is because they provide us with a snapshot that captures what is happening in cells at the moment that they were disrupted to make the extract. But cells are not static entities. At every moment, they are adapting their activities in response to changing combinations of internal and external conditions. Changes in response to any one signal are modified and influenced by the every other condition, within and outside the cell, and understand these complex systems as an integrated whole is the new holy grail of biochemistry.

The aim, then, is to develop models that depict these dynamic interactions within cells, and to understand how such interactions give rise to the properties and behavior that we observe. This is the goal of the emerging field of systems biology that constructs mathematical models and simulations, based on the large data sets generated by transcriptomic, proteomic and other broad-range techniques. Systems biology is truly an interdisciplinary venture, drawing as it does on mathematics and computer science as much as traditional “bench biochemistry”. While the original laboratory techniques of biochemistry are by no means obsolete, they will no longer be the sole tools used to understand what goes on inside of cells.

These newer approaches are already leading to applications that are of tremendous value. Understanding the system level differences between normal and diseased cells can lead to major changes in the way diseases are detected, treated or altogether prevented.

One recent triumph of systems biology has been in an intriguing discovery about how antibiotic drugs work. System level studies of many classes of antibiotics revealed that, regardless of how we think they work to kill bacteria, all of the drugs appear to have a common effect – that of increasing the level of oxidative damage, leading to cell death. This observation suggested that the potency of antibiotics could be enhanced by blocking bacterial responses that protect against oxidation damage. This idea was tested by screening large numbers of compounds for the ability to inhibit a pathway that bacteria use to repair their oxidation-damaged DNA. This screen yielded several compounds, the best of which was able to increase the effectiveness of the drug gentamicin by about a thousand-fold. Such compounds will be of increasing value in a world where antibiotic resistance is on the rise.

BB Wonderland

To the tune of "*Winter Wonderland*"

Milam Hall - It's 12:30
And Ahern's gettin' wordy

He walks to and fro'
While not talkin' slow
Givin' it to B-B-4-5-0

I was happy when the term got started
Lecture notes and videos galore
MP3s got added to my iPod
But recitations sometimes were a bore

And exams bit me roughly
When the curve turned out ugly

I don't think it's so
My scores are too low
Slidin' by in B-B-4-5-0

Final-LY there's an examination
On December 9th at 6:00 pm
I'll have my card packed with information
So I don't have to memorize it then

And I'll feel like a smarty
With my jam-packed note-cardy
Just one more to go
And then ho-ho-ho
I'll be done with B-B-4-5-0

Recorded by David Simmons
Lyrics by Kevin Ahern

Another application of systems biology is in the development of more effective vaccines. Till recently, most vaccines have been developed with little understanding of how exactly they stimulate the immune response. As systems biology approaches give us a better understanding of the changes that vaccines bring about to mediate immunity, it will be possible to identify the patterns that characterize stronger immune responses or adverse reactions to vaccines and even to predict how well particular vaccines

The tert alcohol was a fool
Turned to ketone in making a fuel
The work is complete
The bond gave up heat
Losing all of its family joules

may work in specific populations or individuals. Similarly, system level studies can help identify which drugs might be most effective, with the fewest side-effects, for a given patient, leading to a new era of personalized medicine.

Related to systems biology, and heavily dependent on it, is synthetic biology, which aims to use the knowledge gained from the former to engineer novel biological systems and pathways. Because the technology now exists to synthesize extremely long pieces of DNA, entire genomes can be made synthetically and used to program cells that they are inserted into. It also allows for the possibility of custom-designing an organism to create particular chemical compounds through artificially assembled pathways.

The driver's ed student gave holler
For a driving course worth every dollar
When she passed it today
Her friends had to say
It made her some kind of road scholar

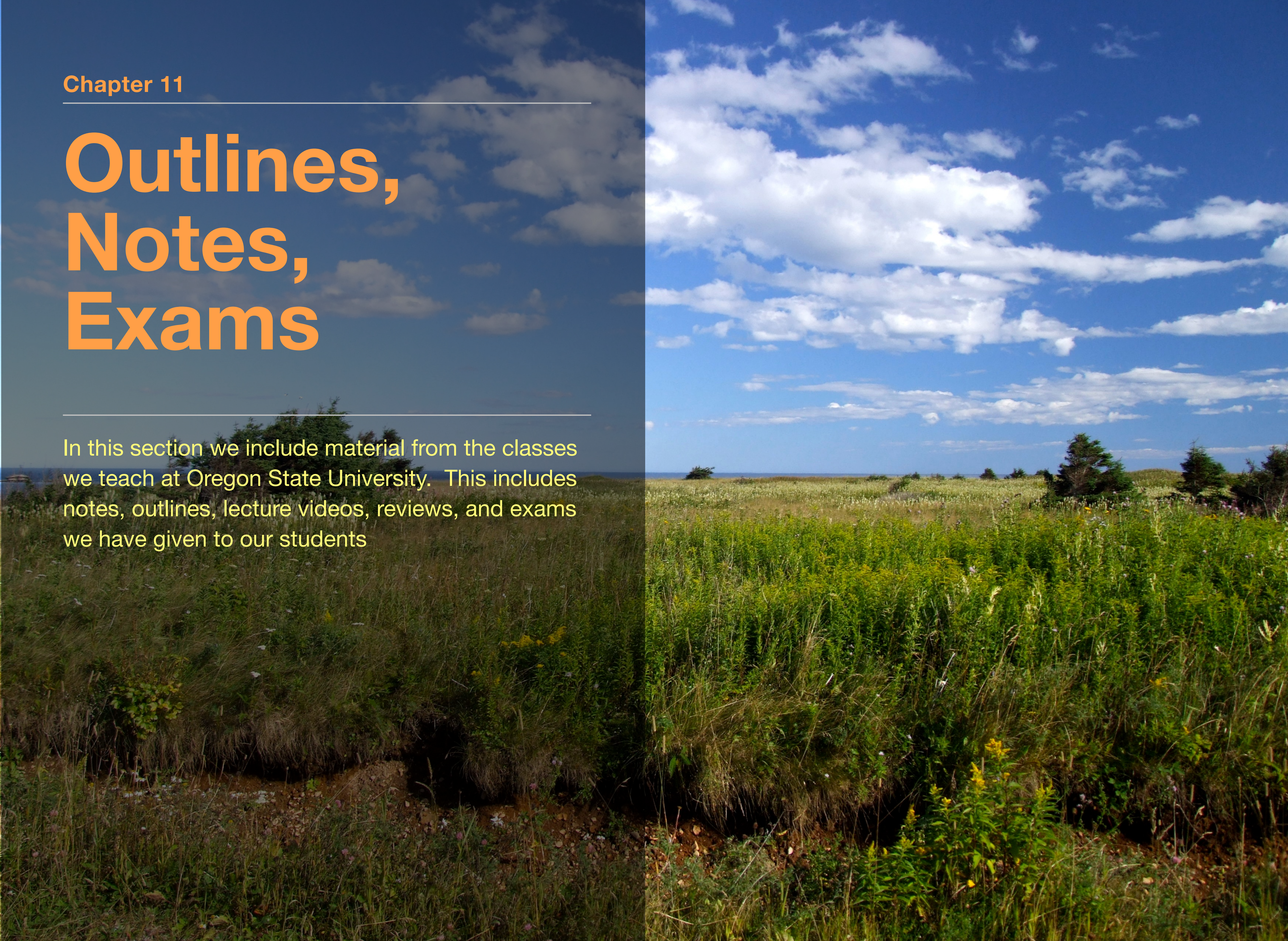
These methods have already been used to produce the drug artemisinin, which is used to treat malaria. The pathway for making a precursor of artemisinin was created by combining a metabolic pathway from yeast with part of another derived from the plant *Artemisia annua*, the natural source of artemisinin. Similar efforts are underway for anticancer drugs, novel drugs, flavoring compounds, etc. One major goal is to create organisms programmed to make biofuels that could potentially replace petroleum.

The successes of systems and synthetic biology, even in their infancy, promise great advances both in our understanding of living systems and in the applications that arise out of that knowledge. The next fifty years in biological research may well eclipse even the amazing accomplishments of the last. The practice of medicine will be transformed. Regenerative medicine will improve, as a better knowledge of stem cells allows us to use them more effectively to replace cardiac muscle lost in a heart attack, neurons damaged in Parkinson's or Alzheimer's, or even to regrow limbs lost in accidents or war. Treatments for our illnesses can be tailored to be optimal for each individual. Biofuels may bail us out when oil supplies run out and engineered organisms may help clean up a polluted planet. And research on longevity may give us the best gift of all- lives extended long enough to witness these advances and to participate in the creation of a new and better world.

The science researcher would whine
At his data darn near every time
When it comes to a graph
There's no cause to laugh
He wonders where he should draw the line

Jump to Chapter

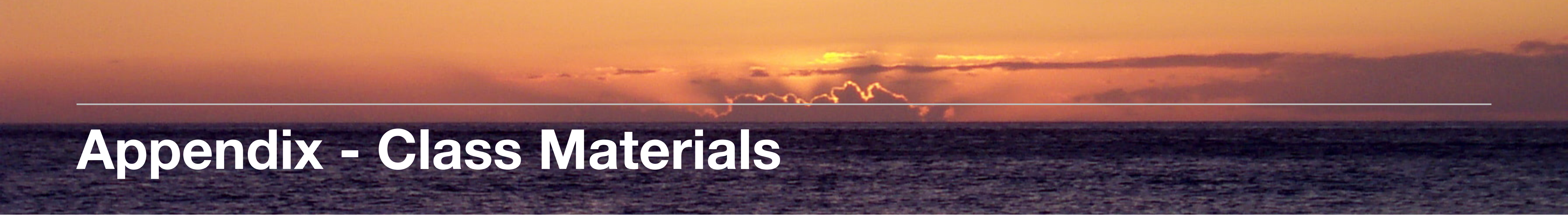
1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12



Chapter 11

Outlines, Notes, Exams

In this section we include material from the classes we teach at Oregon State University. This includes notes, outlines, lecture videos, reviews, and exams we have given to our students



Appendix - Class Materials

APPENDIX 1

This section of the appendix includes links to materials we use to teach our classes. In each case, the hyperlinks to the Outline material and the Videos will take you to an outside Web site, so you will thus need an Internet connection to those materials. The Key Point Summary links lead to relevant sections in Appendix 2.

Appendix I - Class Materials

This section is organized differently from the rest of the text. It is the e-book equivalent of a hybrid Internet/ebook-based appendix. In this section, we place an organized collection of links to videos, online outlines of hyperlinks, and note summaries for the many topics in the first nine chapters.

From BB 450/550

Introduction to Biochemistry / Acids, Bases, Henderson Hasselbalch

Key Points Summary HERE / HERE
Video HERE / HERE /

Protein Structure

Key Points Summary HERE
Video HERE / HERE /HERE

Protein Characterization

Key Points Summary HERE
Video HERE / HERE / HERE

Hemoglobin

Key Points Summary HERE
Video HERE / HERE

Enzymes

Key Points Summary HERE
Video HERE / HERE / HERE / HERE

Catalytic Strategies

Key Points Summary HERE
Video HERE / HERE / HERE

Allostery and Regulation

Key Points Summary HERE

Video HERE / HERE / HERE

Carbohydrate Structures

Key Points Summary HERE

Video HERE / HERE / HERE

Cellular Signaling

Key Points Summary HERE

Video HERE / HERE / HERE

Metabolic Control

Key Points Summary HERE

Video HERE / HERE

Glycolysis & Gluconeogenesis

Key Points Summary HERE

Video HERE / HERE / HERE / HERE

Glycogen Metabolism

Key Points Summary HERE

Video HERE / HERE / HERE

Review Sessions

Video HERE / HERE / HERE

From BB 451/551

Citric Acid Cycle

Key Points Summary HERE

Video HERE / HERE

Lipids and Membranes

Key Points Summary HERE

Video HERE / HERE

Membrane Transport

Key Points Summary HERE

Video HERE / HERE

Mitochondria / Electron Transport

Key Points Summary HERE

Video HERE / HERE / HERE

Photosynthesis

Key Points Summary HERE

Steroid and Lipid Metabolism

Key Points Summary HERE

Video HERE / HERE

Fatty Acid and Prostaglandin Metabolism

Key Points Summary HERE

Video HERE / HERE / HERE

Nucleotide Metabolism

Key Points Summary HERE

Video HERE / HERE

Nitrogen Metabolism

Key Points Summary HERE

DNA Replication, Recombination, Repair

Key Points Summary HERE

Video HERE / HERE / HERE / HERE

Transcription

Key Points Summary HERE

Video HERE / HERE / HERE

Translation

Key Points Summary HERE

Video HERE / HERE / HERE

Gene Regulation

Key Points Summary HERE

Video HERE / HERE / HERE

Sensory Systems

Key Points Summary HERE

Video HERE / HERE / HERE

Review Sessions

Video HERE / HERE / HERE

From BB 350

Introduction

Video HERE

Water/Biochemistry

Video HERE / HERE

Amino Acids/Peptides

Video HERE

Protein Structure

Video HERE / HERE / HERE

Protein Purification

Video HERE / HERE

Enzymes

Video HERE / HERE / HERE

Control of Enzymes

Video HERE / HERE / HERE

Membranes

Video HERE / HERE / HERE

Nucleic Acids

Video HERE / HERE

DNA Synthesis

Video HERE / HERE / HERE

RNA Synthesis

Video HERE / HERE / HERE

Protein Synthesis

Video HERE / HERE / HERE

Biotechnology

Video HERE / HERE

Viruses and Cancer

Video HERE / HERE

Metabolic Energy

Video HERE / HERE

Carbohydrates

Video HERE / HERE

Glycolysis

Video HERE / HERE / HERE

Gluconeogenesis and Glycogen

Video HERE / HERE / HERE

Citric Acid Cycle

Video HERE / HERE

Electron Transport / Oxidative Phosphorylation

Video HERE / HERE

Lipid Metabolism

Video HERE / HERE / HERE / HERE

Photosynthesis

Video HERE

Nitrogen Metabolism

Video HERE / HERE

Other YouTube Video Collections

BB 350 (older - 139 videos) HERE

BB 350 (newer - 36 videos) HERE

Bite Sized Biochemistry (older - 53 videos from BB 450/451) HERE

Comprehensive YouTube Collection (375+ videos) HERE

Kevin also teaches a biochemistry course (BB 100) that has no pre-requisites and is aimed at anyone interested in learning about the subject without technical details. It can be found HERE

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Section 2

Appendix - Key Points

APPENDIX 2

In this section, we include all of the key points of our lectures. Hyperlinks to all of the topics in this section are provided in Appendix 1.

Key Points - Basics

1. Covalent bonds are VERY strong bonds that hold atoms/molecules together. Covalent bonds are the 'glue' that holds together biomolecules.

2. Hydrogen bonds are much weaker bonds that are also important in biological molecules. Hydrogen bonds arise from uneven sharing of electrons between, for example a nitrogen and a hydrogen or an oxygen and a hydrogen. In each case, the hydrogen ends up with a partial positive charge and the other atom has a partial negative charge. The partial positive charge of the hydrogen may be attracted to a partial negative charge on another oxygen or hydrogen. These bonds are weaker than covalent bonds, but are VERY important in stabilizing protein and DNA structures.

3. Water has its relatively high boiling point due to its numerous hydrogen bonds. The double helix of DNA is held together by hydrogen bonds between the individual bases.

4. Hydrogen bonds are additive, so they provide great stability in numbers (as in across a chromosome), but less stability locally (one can easily pull them apart when DNA needs to replicate).

5. Hydrogen bonds are some of the stabilizing forces of proteins. Since protein

The mouse in my house makes me shriek
Every time I espy its physique
The thing that inflames
Me most is his games
He likes to play hide and go squeak

function depends on protein structure, forces that disrupt hydrogen bonds (such as heat) tend to disrupt protein structure and function. It is because of this that cooking food kills bacteria, because it denatures their proteins. Since proteins are the "workhorses" of cells, loss of protein function means loss of cell function, which means death.

6. pH is a measure of the proton concentration in a solution. The pH is the negative log of the hydrogen ion concentration. The lower the pH, the higher the hydrogen ion concentration and the stronger the acid. pH + pOH = 14. The pOH is the negative log of the hydroxide ion concentration. The pK_a is, therefore, the negative log of the K_a. The lower the pK_a for an acid system (such as the acetic acid system discussed in class), the stronger the acid is.

7. Acetic acid (HAc above) is a weak acid, meaning that it doesn't completely dissolve in water. HCl, by contrast is called a strong acid because it completely dissociates in water.

$$HAc <=> H^+ + Ac^-$$

We can write the general equation for the dissociation of ANY weak acid as

$$HA <=> H^+ + A^-$$

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Buffers

1. Weak acids (written as HA) dissociate in water to a limited extent. This ionization can be written as

$$HA <=> H^+ + A^-$$

(where <=> refers to the equiilibrium symbol)

2. For dissociation of such weak acids, the Henderson-Hasselbalch equation applies. It states that

$$pH = pKa + \log \{[A^-]/[HA]\}$$

where the bracketed item (such as $[A^-]$) refers to the concentration of that item.

3. Remember that pH is defined as the negative log of the hydrogen ion concentration

$$-\log[H^+]$$

and the pK_a is defined as the negative log of the acid dissociation constant, K_a

$$-\log(K_a)$$

4. pK_a is a measure of the strength of an acid. The lower the pK_a, the stronger the acid.

5. Very strong acids like HCl that completely ionize in water do not have pK_a values.

6. Buffers are systems of molecules in solution that act to resist pH changes within approximately 1 pH range of their respective pK_a values. For example, acetic acid has a pK_a of 4.76. This means it will be an effective buffer in the range of 3.76 to 5.76.

7. At the pK_a of a buffer, it will have equal amounts of molecules with the appropriate proton and missing the proton. For acetic acid, for example, (abbreviated as HAc), at pH 4.76, it will have equal amounts of HAc and Ac^-. Below pH 4.76, it will have more HAc than Ac^-. Above pH 4.76, it will have more Ac^- than HAC.

8. When referring to a buffer system, we shall use the term 'salt' to refer to the molecule of

the buffer system that has lost a proton. We shall use the term 'acid' to refer to the molecule of the buffer system that has not lost its proton. The difference between a salt and an acid is a single proton.

The corn chippers did not think it neato
When the famous judge came incognito
And marked all their Lays
With a bunch of O.J.s
So that was why Frito banned Ito

9. Adding a strong acid (such as HCl) to a buffer system causes salt (A^-) to be converted into an acid (HA). For every molecule of HCl added, one A^- is converted to one HA. Thus, if one had 500 molecules of HA and 500 molecules of A^- in solution and one added 10 molecules of HCl, the resulting solution would have 510 molecules of HA and 490 molecules of A^-.

10. Conversely, adding a strong base (such as NaOH) to a buffer system causes HA to be converted to A^-. For every molecule of NaOH added, one HA is converted to one A^-. Thus, if one had 500 molecules of HA and 500 molecules of A^- in solution and added 10 molecules of NaOH, the resulting solution would have 490 molecules of HA and 510 molecules of A^-.

11. Molecules can have more than one buffering region. Alanine, for example has both an amine group and a carboxyl group that can gain/lose protons (hydrogen ions). It will thus have two pK_a, one for the carboxyl group and one for the amine group.

12. A buffer system will be at maximum capacity when the concentration of the undissociated acid (HA) equals that of the salt (A^-). That is (Acid = Salt). The Henderson Hasselbalch equation further reveals that when this is true, $pH = pK_a$.

When the hospital beds of last quarter
Didn't fill up the income was shorter
The medical center
Considered some renters
But remained just as doctors without boarders

13. Amine systems (also in amino acids) have two forms:

NH_3^+ and NH_2.

Note that the NH_3^+ is the acid and NH_2 is the salt in our nomenclature.

14. Carboxyl systems have two forms too. COOH has no charge and when it loses its proton, COO^- has a negative one charge.

15. Students make mistakes calling things acids and bases based solely on structure. Consequently, they get confused when they think of an amine like NH_3^+ donating protons (acting like an acid). The same students usually aren't confused, however, about COO^- accepting protons (acting like a base). That is why we avoid calling amine groups "bases" here.

16. The Henderson Hasselbalch equation tells us we can predict the ratio of salt to acid as a function of pH if we know the pK_a. Consequently, we can predict the charge on amino acids in a protein as the pH changes. Subtle changes in pH in the body can have drastic changes in protein structure and function. For example, hemoglobin undergoes drastic changes between the lungs (relatively high pH) and actively respiring tissue (relatively low pH). The pH changes are not very large, so it is important to recognize that very small pH changes can make a big difference.

17. The value of the Henderson Hasselbalch equation is that by knowing the pH and the pKa of a molecule, the approximate charge of it in solution can be determined. For this

book, we will assume that if the pH is more than one unit below the pKa of a group, the proton is ON it. If the pH is more than one unit above the pKa of the group, the proton is OFF it. This is only useful for estimating charge. Determination of the pI (pH at which a molecule has a charge of exactly zero) requires calculation.

The iPhone support staff said “We
Make software that’s always bug-free”
I replied to the jerk
That it just wouldn’t work
Cuz it was an app errant to me

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Protein Structure

1. Protein structure dictates protein function. The structure of a protein is a function of the sequence of amino acids comprising it.

2. Amino acids are the monomeric (building block) units of proteins. They are covalently joined together in peptide bonds to make proteins (polypeptides).

3. There are 20 amino acids commonly found in proteins and of these 20, 19 have a chiral center and thus can exist in two stereoisomeric forms. The only one that doesn't have a chiral center is glycine. Almost all biologically made amino acids are in the same stereoisomeric form - the 'L' form. The 'D' form occurs only in very rare peptides, such as in the cell wall of bacteria (discussed later in the book).

4. Amino acids are grouped into several structural categories based on the composition of their R groups. All biochemistry students should know the names of the 20 amino acids of proteins, which of the groups above each one belongs to and they will need to be able to predict ionization at given pH values if you are supplied pKa values. Of the 20 amino acids in proteins, the ones we will be concerned with have R groups that can ionize. These include the aminos, the carboxyls, the sulfhydryl (cysteine) and the hydroxyl of tyrosine.

5. I mentioned briefly some of the properties of individual amino acids in the lecture. You should know those general properties (such as hydrophobic, hydrophilic, sulfhydryl, and ionizing side chains.) Glycine is the simplest amino acid because it only has a hydrogen as its R-group. It is the only amino acid that doesn't have D and L forms. Another important one structurally is proline.

6. Molecules, such as amino acids, that are capable of gaining/losing more than one proton, have pKa's corresponding to each functional group that can lose a proton. For example, a simple amino acid, such as alanine, has an amino group (pKa about 9.5) and a carboxyl group (pKa about 2.5).

7. A titration plot for a simple amino acid (no R groups that can lose protons) has two flattened regions - each one occurring at the respective pKa. See the problem solving video HERE for more information.

At the North Pole an elf-tracking thing
Uses letters but no numbering
Elf A may be fast
And Elf Z is the last
But Elf S is forever the king

8. A zwitterion is a molecule whose net charge is zero.

9. The pI of a molecule is the point at which its charge is exactly zero. The assumptions above about protons on/off cannot be used to find the pI. It must be calculated. The pI of a molecule is the average of the pK_a values around the point on the titration curve where the charge is zero

10. The sequence of amino acids in a protein is ultimately responsible for all of the properties a protein has. The sequence of amino acids of a protein is referred to as its primary structure.

11. Bonds holding amino acids together in a protein are called peptide bonds and they occur between the alpha amino group of one amino acid and the alpha carboxyl group of the next one.

12. Peptide bonds form resonant structures such that the bond itself behaves like a double bond. Double bonds cannot rotate (unlike single bonds) and thus they define a plane. Alpha carbons on either side of a peptide bond are generally arranged in a *trans* configuration (about 10,000 *trans* to one cis), except when proline is involved. Peptide bonds involving proline favor the *trans* by about a 100 to 1 ratio.

In the shuttle it seems to confound
The astronauts high o'er the ground
Every time when they read
It's almost guaranteed
They can't ever put their books down

13. The bonds around the alpha carbon (the carbon bonded to both an amino group and a carboxy group) can both rotate, however, because they are single bonds. One can thus describe a polypeptide as a series of planes separated by an alpha carbon, with the planes each being rotated a certain number of degrees relative to the alpha carbon. If we think of the alpha carbon as being in between two planes, then the plane on the left can rotate (theoretically) 360 degrees and the plane on the right can also theoretically rotate 360 degrees. These angles of rotation of planes are referred to as phi and psi angles. Phi refers to the rotational angle around the single bond between the alpha amino group and the alpha carbon. Psi refers to the rotational angle around the single bond between the alpha carbon and the alpha carboxyl group.

14. In the early 1960s, G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan recognized that not all rotations of phi and psi would be theoretically feasible because steric hindrance would preclude some rotational positions. They plotted theoretical rotations of psi versus phi and calculated which of these angles would provide stable structures. The resulting plot came to be known as a Ramachandran plot. In it, the regions of predicted stability turn out to be regions of known stability from protein structures that have been determined.

15. The secondary structure of a

polypeptide refers to regular/repeating structure(s) arising from interactions between amino acids that are relatively close together in primary sequence. This means less than 10 amino acids away.

In St. Louis the planners could see
That their Gateway might not come to be
The people opposing
The structure imposing
Saw it as their arch enemy

16. One protein secondary structure that is stable in both real proteins and theoretical ones (Ramachandran plots) is the alpha helix. Alpha helices are one type of secondary structure and form coils.

17. Hydrogen bonds are primary forces stabilizing secondary structures. In alpha helices, carbonyl oxygen from a peptide bond forms a hydrogen bond with an amine nitrogen of another peptide bond four amino acids distant.

18. Certain amino acids with simple side chains, such as alanine, are very favorable for formation of alpha helices, whereas bulky (tryptophan) or cyclic (proline) amino acids tend to disrupt alpha helices. Thus, one can reasonably accurately predict from an amino acid sequence which regions of a protein sequence will exist as alpha helices and which will not.

19. Another type of common secondary structure commonly found in protein is the beta strand (note that the term beta sheet refers to layering together of beta strands together), which consists of amino acid backbones in a V shape (like the pleats of a drape). A beta strand can be thought of as a helix in two dimensions though that is an over-simplification. Again, specific amino acids favor the formation of this structure and others (proline again) favor its disruption.

Out at State Farm the agent extends
A payment to tie up loose ends
This brings to a close
What the owner now knows
The way that a Mercedes bends

20.Beta sheets arise from arrangement of beta strands. These arise from interactions (hydrogen bonds) between beta strands (parallel or antiparallel), such that the carbonyl oxygen of one side interacts with the amine hydrogen with the other. Beta strands can also orient their R groups such that they interact appropriately (hydrophobic-hydrophobic, for example). Beta sheets can be oriented in several ways.

21. Essential features of proteins that are essential for their overall structure are turns. Turns interrupt secondary structure (alpha helices and beta strands) and often involve proline and glycine residues.

22. Another type of fibrous protein is collagen, the most abundant protein in your body. It contains three intertwined helices comprised of abundant repeating units of glycine, proline, and hydroxylproline

23. Hydroxylation of proline is a post-translational modification (occurs after the protein is made) and the hydroxyls are placed there in a reaction that uses vitamin C.

24.The hydroxyls of hydroxyproline can react with other, forming covalent cross-links that make the collagen fibers more sturdy.

25.Tertiary structure relates to interactions between amino acids in a protein that are

When at bedtime the kid was a pest
Mom and Dad only had to suggest
If he just wouldn’t stop
They would go get a cop
And then charge with resisting a rest

not close in primary sequence. These interactions are made possible by folding of the protein chain to bring the distant amino acids closer together.

26. Tertiary structure is stabilized by disulfide bonds, ionic interactions, hydrogen bonds, hydrophilic, and hydrophobic interactions. Disulfide bonds are the strongest forces holding tertiary structure together, as they are covalent bonds.

27. Most proteins that are in cells are globular in nature.

28. Myoglobin is a protein that acts as an oxygen 'battery', storing oxygen in muscles for when it is needed. Myoglobin contains a heme group that contains iron. Heme is a 'prosthetic group', which refers to a non-amino acid containing group that binds to a protein and augments its function.

29. Amino acid residues in myoglobin are arranged such that hydrophilic amino acids are arranged on the outside and hydrophobic amino acids are largely arranged on the inside.

30. Porin is a membrane protein. Proteins embedded in membranes often have external amino acids that are hydrophobic so they can interact with the non-polar portions of membranes. Porin, in addition, has a hole in the center that allows water to pass through it. The amino acids in porin are arranged with non-polar amino acids outside and polar amino acids inside.

31. Quaternary structure of proteins relates to the interactions between separate polypeptide chains within the protein. The word polypeptide refers to a polymer of amino acids. A protein may contain one or more polypeptides, is folded and may be covalently modified.

32. Hemoglobin (and many other proteins) have multiple polypeptide subunits. Interactions between the subunits include disulfide bonds, ionic interactions, hydrogen bonds, hydrophilic, and hydrophobic interactions. Modification of the quaternary structure of a protein may have the same effects as modification of its tertiary structure - alteration of its function/activity.

33. Folding is necessary for proteins to assume their proper shape and function. The instructions for folding are all contained in the sequence of amino acids, but we do not yet understand how those instructions are carried out rapidly and efficiently. Levinthal's paradox illustrates the fact that folding is not a random event, but rather based on an ordered sequence of events arising from the chemistry of each group.

34. Proper folding of a protein is essential. Cells have complexes called chaperonins that help some proteins to fold properly. Misfolding of proteins is implicated in diseases, such as mad cow disease and Creutzfeld-Jacob disease in humans. The

At the dairy, Ms Margo R. said
Looking up where they make the cheese spread
“I don’t get the jokes
Of these curd forming folks
I guess it’s whey over my head”

causative agent in these diseases is a "contagious" protein that is coded by the genome of each organism. When it doesn't fold properly, it helps induce other copies of the same protein to misfold as well, resulting in plaque-like structures that destroy nerve cells.

35. Hemoglobin (and many other proteins) have multiple polypeptide subunits. Interactions between the subunits include ionic interactions, hydrogen bonds, and hydrophobic interactions. Modification of the quaternary structure of a protein may have the same effects as modification of its tertiary structure - alteration of its function/ activity.

36.The enzyme ribonuclease (RNase) is interesting in being very stable to heat and other things that denature/inactivate other proteins. (By the way, denaturation is a word that means the tertiary and/or quaternary structure of a protein is disrupted.). RNase has disulfide bonds that help it to remain resistant to denaturation.

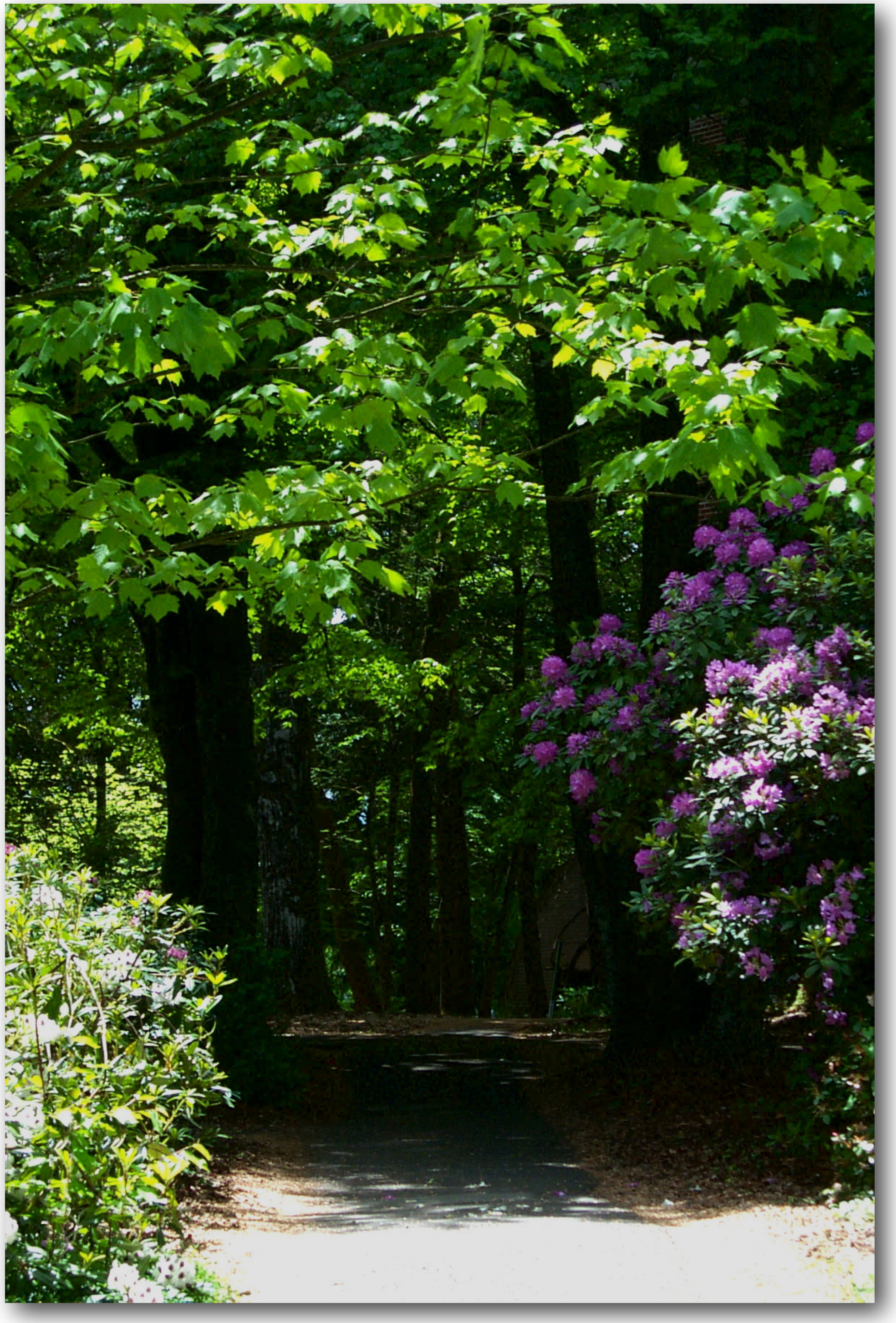
37. Some chemicals, such as mercaptoethanol, can reduce the disulfides (between cysteine residues) in proteins to sulfhydryls. In the process of transferring electrons to the cysteines, the sulfhydryls of mercaptoethanol become converted to disulfides. Treatment of RNase with mercaptoethanol reduces RNAse's disulfides to sulfhydryls. Subsequent treatment of RNase with urea disrupts hydrogen bonds and allows the protein to be denatured.

38. Interestingly, removal of the mercaptoethanol and urea from the solution allows RNase to refold, reestablish the correct disulfide bonds, and regain activity. Clearly, the primary sequence of this protein is sufficient for it to be able to refold itself to the proper configuration.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Macromolecular Characterization



1. Proteins sometimes have amino acids in them that are chemically modified. Chemical modification of amino acids in proteins almost always occurs AFTER the protein is synthesized (also described as post-translational modification). Examples include hydroxyproline and hydroxylysine in collagen, gamma carboxyglutamate in prothrombin, and phosphoserine in many other proteins. Modification of the collagen residues allows for the triple helical structure of the protein and for the strands to be cross-linked (an important structural consideration).

2. Purification of proteins exploits differences in charge, size, shape, and affinity for specific compounds. Centrifugation (artificial gravity) provides a means of precipitating cellular components. The amount of centrifugal force applied (speed of spin) determines the rate with which a given substance will be driven towards the bottom of the tube. The largest components will precipitate the fastest at the slowest speeds. The smallest components will precipitate the slowest and may remain in solution, allowing one to fractionate materials based on size and solubility in water.

3. Dialysis provides a means of separating large molecules (like proteins, DNA, etc.) from small molecules (like salts, etc.) by encasing the protein/salt mixture in a membrane. The membrane has holes (pores) big enough to let small molecules to pass through it, but too small to let out the big molecules. Thus, with this technique, one can effectively remove the salt from a protein.

4. Gel filtration (gel exclusion chromatography) provides another way to separate large molecules from smaller ones. It employs beads with tunnels in them. The beads are packed in a column. The beads have buffer running through them and the openings to the tunnels are a fixed size (called an exclusion limit). The size of the opening determines the maximum size of molecule that can enter it. Molecules smaller than the exclusion limit

A Chinese composer named Ong
Wrote a beautiful lyrical song
Except he did make
A single mistake
That he would never change, writer Ong

enter the beads and travel a longer path than molecules larger than the exclusion limit. Thus, when large and small proteins are applied to a column, the large proteins come through first and the small ones come last.

5. Ion exchange chromatography uses beads in a column also, but instead of tunnels, the beads are coated with a molecule having either a positive or negative charge. If a mixture of molecules with positive and negative charges is added to the column, the negative molecules will "stick" to the column when the beads are coated with positive charges. The molecules with positive charges will not stick to the positively charged beads and thus one can separate positive and negative molecules using a column containing beads with only one type of charge.

6. Affinity chromatography exploits the tendency of many proteins to 'bind' to molecules. For example, many proteins bind ATP. If one takes beads and coats them with ATP and then passes a protein mixture through the column, only those proteins that bind to ATP will stick to the column. The others will pass through it freely. With affinity chromatography, one can separate proteins that bind a specific molecule from proteins that don't bind that molecule.

7. HPLC (High Performance Liquid Chromatography) employs densely packed columns containing material with chemical groups on them that interact with molecules as they are pumped through the column. The most popular type of HPLC is reverse phase chromatography, which uses column material that is very non-polar. Hydrophobic interactions cause the less polar molecules to be retained the longest by the column, whereas the polar molecules have no affinity for the column material and pass through first.

8. Gel electrophoresis is a method for separating molecules using an electric field across a support (gel). In this method, molecules separate by size. The smallest ones move the fastest and the largest ones move the slowest.

9. Isoelectric focusing is a technique that separates molecules on the basis of their pI (pH at which their net charge is exactly zero). It is performed in tubes containing special compounds (polyelectrolytes) that migrate to specific points in the tube when in the presence of an electric field. This effectively creates a pH gradient from one end of the tube to the other. If proteins are added to the tube as the gradient is getting established, they will migrate to the point in the tube where the pH corresponds to their pI and they will migrate no further, since they will have a charge of zero.

10. 2D gel electrophoresis is a powerful tool for proteomics that combines the techniques of isoelectric focusing with SDS-PAGE. In this method, proteins are first separated according to their pI values by isoelectric focusing. Then the tube from the isoelectric focusing is applied to the top of an

The octuplets' mother and dad
Reflected on children they had
Their bundles of joys
Make ear piercing noise
So now they are stork raving mad

SDS-PAGE gel and the proteins are separated by size. The result is a two dimensional separation of virtually every protein in the cell.

11. Nucleic acids (which are negatively charged and 'rod-like' in shape) can be separated by agarose gel electrophoresis readily. In this technique, a 'gel' is made that consists of a matrix material (agarose) that forms a sort of 'mesh' of hole through which the DNA molecules pass. Electric fields are used to separate macromolecules by size. The sample is loaded on the top of the gel and electrical current is passed through it such that the bottom electrode is positive and the top one is negative. Negatively charged DNA molecules at the top of the gel are driven away from the top towards the bottom. Small molecules make their way through the gel fastest and big molecules travel more slowly. The key to gel electrophoresis is to have all of the molecules being separated have a negative charge.

12. Proteins, however are usually globular in their native state and, without other modification, may be negatively, positively OR neutrally charged. Another type of electrophoresis employs polymers of acrylamide (called polyacrylamide) to form the mesh of the gel. This mesh has smaller holes that agarose and allows separation of smaller molecules like proteins. To separate proteins on the basis of size, the detergent sodium dodecyl sulfate (SDS) is add to the protein mixture, causing the proteins to denature, assume rod-like shapes, and be coated uniformly with the negative charge of the SDS. Consequently all proteins in the mixture obtain a negative charge and can be separated just like DNA on SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

At the plant singles bar for a while
The anther developed a smile
It was clear as a crystal
He adored every pistil
When he told them that “I like your style”

I don’t have a good memory
Of the things that I’ve done recently
I am still at a loss
For the boom’rang I tossed
Perhaps it will come back to me

13. During purification of proteins it is important to follow the purification process. At each step of the process, a small sample of the protein extract is taken and the total amount of protein, and the amount of activity of the desired protein are measured. The specific activity of the protein in the tube is the amount of activity (in units) divided by the total mass of protein. The yield of the desired protein at any point in the purification process is the number of units of the desired protein at that point divided by the number of units that one started with. The purification level at any point is the specific activity at that point divided by the specific activity one started with.

14. Breaking large proteins down into smaller pieces is also important for studying them. Some reagents include cyanogen bromide (breaks bonds on carboxyl side of methionine residues in a protein) and enzymes known as proteases. One such protease is trypsin, which cleaves on the carboxyl side of lysine and arginine residues in a polypeptide. Thrombin is another enzyme that breaks peptide bonds. It cleaves them on the carboxyl side of arginine in a polypeptide.

15. Immunological techniques aid in identifying specific proteins. Antibodies (immune system proteins) recognize and bind to specific structures. The structures antibodies bind to are called antigens. Usually antibodies are targeted against specific protein structures. Since proteins differ from each other in their structure, molecules (like antibodies) that bind to specific structures will bind to specific proteins.

16. Antibodies are very useful in that they can be linked to fluorescent dyes, gold particles, or enzymes to help one visualize where an antibody has bound. They are useful in binding to specific cellular structures (*in situ* hydridization), to identify where within a cell or within a tissue a particular protein is located.

17. A technique that employs antibodies is western blotting. In this technique, a mixture of proteins is separated by SDS-PAGE. The proteins in the gel are transferred and bound directly to a membrane, which is then treated with an antibody specific for one of the proteins. The membrane is washed to release unbound antibody and then the antibody-protein complex is visualized. This can be by a fluorescent technique or more commonly by an enzyme linked to the antibody as above. In any case, the protein of interest is identified in this way.

18. MALDI-TOF is a mass spectometric analysis instrument that facilitates determination of polypeptide masses with great accuracy. It employs a laser, which, when activated, causes a polypeptide sample to volatilize in the evacuated chamber of the instrument. Volatilization causes the molecules to both ionize and to break at peptide bonds. Masses are determined by the length of time it takes for the ions to travel through the chamber to the detector. The time it takes them to make the transit (Time of Flight= TOF) is proportional to their mass. Smaller fragments move faster than larger fragments. Using MALDI-TOF, it is possible to determine the sequence of amino acids in a polypeptide based on the analysis of the masses of the various fragments that arise from ionization.

19. X-ray crystallography is a technique whereby a crystal of a pure protein (or DNA or RNA) is subjected to beams of x-rays. The movement of x-rays through the crystal is affected by their interaction with electron clouds, causing the x-rays to be bent or deflected. By studying the angles of deflection, one can, using sophisticated computer analysis, determine the electron density of the material the x-rays passed through. The electron density map gives 3D coordinates of every atom in the crystal to within a few Angstroms.

Key Points - Hemoglobin

1. Myoglobin and hemoglobin are related proteins involved in (respectively) storing and carrying oxygen in the body.

2. Myoglobin is a single subunit protein with high affinity for oxygen. It holds oxygen tighter than hemoglobin and serves in a battery-like capacity in tissues to release oxygen when tissue oxygen concentration is very low. Myoglobin can take oxygen from hemoglobin.

A worried young scientist reckons
He must do something when aging beckons
So he'll make himself younger
By developing hunger
So then he can go back four seconds

3. Hemoglobin is a four-subunit protein complex (two alpha subunits and two beta subunits) that serves to carry oxygen from the lungs to the tissues where it is needed. Hemoglobin is genetically related to myoglobin and is evolutionarily derived from it.

4. Myoglobin and hemoglobin both have porphyrin rings (like in chlorophyll) to hold ferrous (Fe++) iron. The specific porphyrin in these proteins is Protoporphyrin IX. Ferrous iron is the form of iron involved in carrying oxygen. Ferric iron (Fe+++), which is the oxidized form of iron will not carry oxygen. Heme is a term used to describe the protoporphyrin IX complexed with iron.

I do not think it is a good sign
I'm so busy that I must decline
The demand of my boss
To get the watch that she lost
Because I just can't find the time

5. The iron in hemoglobin and myoglobin is held in place by five molecules - four nitrogens of the protoporphyrin IX ring and a histidine (called the proximal histidine). Oxygen is carried between the iron and an additional histidine (called the distal histidine) not involved in holding the iron.

6. If one plots the percentage of oxygen sites bound versus partial pressure for myoglobin, a hyperbolic curve is generated, consistent with a molecule with a single binding site and a high affinity for oxygen. The P_{50}, which is the partial pressure of oxygen necessary to fill 50% of the myoglobins with oxygen, is very low for myoglobin, consistent with high affinity. Because myoglobin has high affinity for oxygen, it doesn't release much oxygen until it is in an environment with very low oxygen pressure. For this reason, it would be a poor oxygen transport protein.

7. Hemoglobin is much better designed to meet an organism's physiological needs for transporting oxygen than myoglobin. This is due to its four-subunit organization (one heme per subunit and one oxygen carried per subunit) which behaves in a cooperative fashion in binding oxygen.

8. Binding of oxygen by the iron atom causes it to be pulled 'up' slightly. This, in turn, causes the histidine attached to it to change position slightly, which causes all the other amino acids in the subunit to change slightly. The changes in shape (different 'states') result in the protein gaining affinity for oxygen as more oxygen is bound. The phenomenon is referred to as cooperativity.

9. Hemoglobin can exist in a "tight" state, called 'T', which exhibits low oxygen binding affinity. Hemoglobin in the T state will tend to release oxygen.

10. A second state of hemoglobin is the "relaxed" or R state, which exhibits increased oxygen binding affinity. Binding of oxygen by hemoglobin helps it to "flip" from the T to the R state and release of oxygen by hemoglobin helps it to flip from R to T.

11. A molecule called 2,3-bisphosphoglycerate (2,3 BPG) is produced by actively respiring tissues. It can bind in the gap in the center of the hemoglobin molecule and in doing so, stabilize the T state and favor the release of oxygen. Thus, tissues that are actively respiring get more oxygen. 2,3 BPG is noteworthy because the blood of smokers has a higher concentration of the molecule than the blood of non-smokers.

12. Fetal hemoglobin differs from adult hemoglobin in having two gamma subunits in place of the two beta subunits that adults have. This changes the hemoglobin molecule such at 2,3BPG can't bind, so fetal hemoglobin exists more in the R state and has a higher affinity for oxygen than adult hemoglobin. Thus, the fetus can take oxygen from the mother's hemoglobin, due to this property. The fetus won't release oxygen as readily as the adult, but it doesn't have a high oxygen need, due to not exercising heavily.

13. The Bohr effect describes physiological and molecular responses to changes in pH with respect to oxygen and carbon dioxide in the body. The oxygen effects arise from changes in the tertiary structure of hemoglobin arising from binding of protons to histidines in the molecule when under low pH.

14. Rapidly metabolizing tissues (such as muscle) generate lower pHs, due to release of carbon dioxide and its conversion to carbonic acid by carbonic anhydrase. Carbonic acid readily

loses a proton, becoming bicarbonate.

15. Thus, rapidly metabolizing tissues generate protons, which get absorbed by hemoglobin, which releases oxygen to feed the tissues.

16. CO_2 can also be taken up by hemoglobin at amine residues, causing protons to be released. Note that CO_2 binds hemoglobin at a site other than what oxygen binds. CO, however, can compete with oxygen for binding to the heme.

17. In the lungs, a reversal of this process occurs. Remember that the oxygen concentration in the lungs is high, so oxygen forces off the carbon dioxide and the carried protons from the hemoglobin. Addition of a proton to bicarbonate re-creates carbonic acid, which undergoes the reversal of the earlier carbonic anhydrase reaction, causing CO_2 to be released to the lungs as a gas.

18. Sickle cell anemia is a genetic malady that results in a hemoglobin that polymerizes under low oxygen conditions, causing blood cells to form sickle shapes. These can get stuck in capillaries (painful) and the cells can be removed by the body as possibly damaged, resulting in anemia.

19. Sickle cell anemia appears to provide protection against malaria.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Enzymes

1. Enzymes are proteins that catalyze reactions.

2. Enzymes are capable of speeding reactions quadrillions of times faster than the same reactions would occur in the absence of enzymes.

At the hamburger stand there was one
Young lady who did an end run
Around regulations
And caused a sensation
By putting her hair in a bun

3. Non-proteinaceous molecules that bind to enzymes and help the enzymes to catalyze reactions are called coenzymes.

4. The Gibbs free energy is the energy available to do useful work in reactions. The change in the Gibbs free energy for a reaction is important because it determines whether a reaction is favored ($\Delta G < 0$), unfavored ($\Delta G > 0$), or at equilibrium ($\Delta G = 0$).

5. Thus, when the Gibbs free energy change is negative, the reaction in question goes forward as written, but when the Gibbs free energy change is positive, the reaction goes in reverse.

6. A related term to ΔG is $\Delta G^{\circ\prime}$, which is the Standard Gibbs Free Energy change. This refers to the Gibbs Free Energy change for a reaction under standard conditions. Since most reactions occur at non-standard conditions, ΔG is much more useful than $\Delta G^{\circ\prime}$. In fact, the sign of $\Delta G^{\circ\prime}$ does NOT tell the direction of a reaction, except under standard conditions.

In choosing a theme for the rowers
The adman gave what they asked for
A clever cliché
He inverted to say
"No ifs, ands or buts, just oars"

7. Chemical reactions require activation energy (we'll call it ΔG^{+} here) in order to get started. Catalysts (both enzymes and non-biological catalysts) act by lowering ΔG^{+}. Catalysts DO NOT CHANGE ΔG. All they do is lower the energy required to activate the reaction. While enzymes speed reactions immensely, they therefore DO NOT CHANGE THE OVERALL REACTION CONCENTRATION AT EQUILIBRIUM. They simply allow the reaction to get to equilibrium faster.

8. ΔG is affected by the concentration of reactants and products of a reaction by the following equation

$$\Delta G = \Delta G^{\circ\prime} + RT\ln\{[\text{Products}]/[\text{Reactants}]\}$$

(Note, this assumes a single reactant and a single product)

Thus, as product concentrations increase, the ΔG will become more positive.

9. If one performs an experiment in which a fixed amount of enzyme is added to 20 different tubes, each containing a different amount of substrate (molecule that the enzyme catalyzes the reaction on) and then lets the reaction in each tube go for a fixed amount of time, one will create varying amounts of product when the tubes are analyzed. The greatest amount of product will be found in the tube which had the greatest amount of substrate. If one measures the concentrations of each product and divides by the time the reaction occurred, one obtains a velocity for each reaction. A plot of the velocity versus the substrate concentration (V versus S) from the experiment looks like the binding curve of myoglobin for

The cement man complained with great ardor
To his boss, “I don’t think it’s smarter.
Everything that I do
To satisfy you
Just keeps getting harder and harder.”

oxygen - hyperbolic.

10. Velocity of an enzymatic reaction is measured as the concentration of product formed per time. Maximum velocity (V_{max}) occurs in a reaction when the enzyme is saturated with substrate. V_{max} depends on the amount of enzyme used to measure it.

11. In contrast to V_{max}, K_{cat} is a constant for an enzyme. It is also known as the turnover number and corresponds to the number of molecules of product made per molecule of enzyme per time (usually in seconds). 1000/second means 1000 molecules of product per molecule of enzyme per second.

12. We define K_m as the substrate concentration that gives $V_{max}/2$. Whereas the V_{max} varies, depending on the amount of enzyme that one uses, the K_m is a constant for a given enzyme for its substrate.

13. The higher the K_m of an enzyme, the LOWER its affinity for its substrate. This is because a high K_m means that it takes a LOT of substrate before the enzyme gets to $V_{max}/2$. K_m is frequently referred to as the affinity of the enzyme for a substrate, though that is not 100% correct. Nevertheless, we say that a high K_m is consistent with a low affinity of enzyme for substrate and conversely, that a low K_m is consistent with a high affinity of enzyme for substrate.

14. If one lets a reaction go for a long time, it will reach equilibrium. At equilibrium, the relative concentration of products and reactants do not change. Initial velocities of reactions are therefore measured so as to avoid allow the product to accumulate and favor the reverse reaction.

15. Lineweaver-Burk plots are alternative plots of V vs S data obtained by taking the inverse of each and plotting it, thus making a 1/V vs 1/[S] plot (also called a double reciprocal plot).

16. On a Lineweaver-Burk plot, the Y intercept is $1/V_{max}$ and the X intercept is $-1/K_m$.

17. The catalytic actions of enzymes appear to be related to their ability to be at least slightly flexible. Originally, Fischer proposed a model of catalysis called the Lock and Key model. It described enzymes as inflexible and the substrate as like a key fitting into a lock. While substrates do, in fact, fit into enzymes somewhat like a key, the enzyme is NOT rigid.

18. Koshland's model of enzyme action, called the Induced Fit model says that not only does the enzyme change the substrate (via catalysis), but the substrate also changes the enzyme shape upon binding. This transient change of enzyme shape is important for catalysis because it may bring together molecular groups (such as a phosphate and a sugar) that may not be close together in the enzyme prior to the change in enzyme shape.

Remember that enzymes also work by orienting substrates together in the proper way to maximize their

Perhaps it was heavenly love
Or some coincidences thereof
That gave Matt a ‘B’
In trigonom’try
He thinks ‘twas a sine from above

likelihood of bouncing together in a way that leads to making a bond.

19. Chemical changes brought about by catalysis facilitate a last change in enzyme shape to allow for the release of the products. When this happens, the enzyme returns to its original shape and remains unchanged by acting to catalyze the reaction.

When the pant leg got torn to extremes
Its owner was left without jeans
A seamstress could make
A new leg in a shake
Or perhaps just at least sew its seams

20. "Perfect" enzymes are enzymes that have evolved to the point where any additional mutation will reduce their ability to catalyze reactions. They are not common. Perfect enzymes have a very high ratio of K_{cat}/K_m and are such that the only thing that inhibits their ability to function more efficiently is the rate of diffusion of substrate in water.

21. The active site of an enzyme is the place in the enzyme where a reaction is catalyzed. A substrate is a molecule bound by an enzyme and acted upon it.

22. Substrate binding to enzymes is relevant to catalysis that we will consider. The first is the category of Sequential Displacement. It has two subsets.

    a. Random binding - the order of binding multiple substrates is not rigidly set.

    b. Ordered binding - Simple ordered binding - one substrate binds first followed by another followed by release of product.

23. Note that the models of substrate binding above are both non-covalent.

24. Another non-covalent enzyme mechanism involving multiple substrates is called Double Displacement (or Ping-Pong kinetics). In this method, the enzyme only binds one substrate at a time, but switches back and forth between different states. In the exam given in class, transaminase flips back an forth between carrying an oxygen or carrying an amine group. This also affects what substrate it binds to.

25. Allosterism is a phenomenon in which a small molecule interacts with a protein and affects the protein’s activity. Such an enzyme is an allosteric enzyme. There is similarity between the kinetics of allosterically acting enzymes and the cooperativity of binding of oxygen by hemoglobin.

The tert alcohol was a fool
Turned to ketone in making a fuel
The work is complete
The bond gave up heat
As it lost all its family joules

26. Enzymatic reactions can be inhibited by reversible and irreversible processes. Reversible processes involve binding of an inhibitor and its subsequent release. Irreversible processes generally involve covalent attachment of a molecule to an enzyme followed by its inactivation.

27. Competitive Inhibition - This type of reversible inhibition occurs when the inhibitor competes with the substrate for the binding site on an enzyme. The greater the concentration of inhibitor, the greater the inhibition. However, competitive inhibition can be overcome by increasing amounts of substrate. The apparent V_{max} of competitive inhibition does not vary from the V_{max} of the same reaction when uninhibited. The apparent K_m, however, does vary, because it requires more substrate to get the same velocity as the uninhibited reaction.

28. Non-competitive inhibition - This type of reversible inhibition occurs when an inhibitor binds to an enzyme at a site unrelated to the substrate binding site. In this case, the inhibitor's binding to the enzyme is unrelated to the binding of the substrate and the inhibitor does not have to have a structure like that of the substrate. Thus, the inhibitor and substrate don't compete with each other. The inhibitor can inhibit the enzyme (during its binding) without interference from the substrate. Therefore, increasing substrate concentrations cannot eliminate the effect of the inhibitor. In this case, the Vmax is lowered, but the Km is unchanged.

29. Students should be able to depict or understand graphically (V vs S, Lineweaver-Burk) what occurs in competitive and noncompetitive inhibition.

30. Chemicals, such as DIPF and iodoacetate, covalently (and irreversibly) bind to the side chains of specific amino acids (serine and cysteine, respectively) and if these side chains are essential for the catalytic action of the enzyme, the enzyme may not catalyze reactions after being treated with these chemicals.

31. Penicillin is a substance that resembles the substrate of an

At the circus it is common sense
If a fire breaks out your defense
Should be to be scopin'
The places most open
Because heat will be most in tents

Politicians may find it profound
But we hope that they will come around
To this simple advice
For keeping things nice
Throwing dirt means you’re just losing ground

enzyme in bacteria that helps make the bacterial cell wall. When it binds to the enzyme, it inactivates the enzyme by covalently bonding to the active site, thus destroying the enzyme (and killing the bacterium containing it). An inhibitor of this type is known as a suicide inhibitor.

32. There are six classes of reactions catalyzed by enzymes. They include Oxidation-Reduction Reactions (electrons gained/lost), Ligation Reactions (two molecules put together), Isomerization Reactions (intramolecular rearrangements), Group Transfer Reactions (movement of a part of one molecule to another molecule), Hydrolytic Reactions (breakdown reactions using water), and Lyases (breakdown reactions involving a double bond, but not using water to break the molecule down).

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Catalytic Strategies

1. Proteases catalyze the hydrolysis of peptide bonds in polypeptides. They are usually fairly specific for certain amino acids and cut at or near those amino acids.

2. Chymotrypsin is a protease whose activity has been closely studied. Conveniently, the activity of chymotrypsin can be studied using an artificial substrate which, when cleaved by the enzyme, releases a yellow product.

3. When the release of the colored substrate by the enzyme is studied, it appears to occur in two different rates. First there is a VERY rapid release of the colored substrate. After that initial burst of activity, the remaining yellow color is released slowly.

4. The reason appears to be that the reaction catalyzed occurs in two steps. The first step cleaves the bond to produce the yellow product, which is rapidly released. The other product of this reaction is the remainder of the substrate that is covalently linked to the enzyme. In order for the enzyme to bind another substrate molecule and release more yellow color, it must first release the covalently bound molecule. This step occurs slowly and explains why subsequent yellow molecules are released slowly - after the initial one is released, the enzyme must remove the covalently bound molecule, bind a new substrate, and cut the substrate and the continue the process repeatedly.

5. Chymotrypsin is an example of a protease that employs reactive serine in its active site. Such an enzyme is called a serine protease. Treatment of chymotrypsin with DIPF, which covalently links to serines, inactivates the enzyme.

6. Serine proteases form covalent intermediates with their polypeptide substrates. The first step involves nucleophilic attack of an alkoxide ion on the polypeptide substrate to form an acyl-enzyme intermediate. Formation of this intermediate results in cleavage of the peptide bond and release of the first polypeptide fragment. The acyl-enzyme intermediate is

resolved by addition of water to release the other portion of the original polypeptide along with regeneration of the original enzyme active site. This last step occurs relatively slowly.

7. In the active site of chymotrypsin (and other serine proteases) is a so-called catalytic triad of amino acids that includes a serine hydrogen bonded to a histidine. The histidine is, in turn, hydrogen bonded to an aspartic acid residue in the active site. Each of the hydrogen bonds of the catalytic triad is important in the catalytic mechanism. The nucleophilic alkoxide ion on serine is made possible ultimately by interactions in the catalytic triad and these hydrogen bonds.

8. The catalytic triad is not unique to chymotrypsin. The serine protease known as subtilisin also has the same catalytic triad and employs a similar mechanism.

9. Besides the catalytic triad, the enzyme has two other important sites to consider. The first is the oxyanion hole that stabilizes a tetrahedral intermediate that arises during the catalysis. The second, called as S1 pocket, is where the substrate binds. Both the oxyanion hole and the S1 pocket are adjacent to the active site (catalytic triad).

I confess with a bit of hostility
I succumbed due to some gullibility
My info got took
By a mean online crook
Possessing a great lie ability

10. Many other proteases and other enzymes share similarities with the mechanism used by serine proteases. Other proteases include cysteine proteases (use cysteine and histidine in the active site), aspartyl proteases (use aspartic acids and water in the active site) metalloproteases (use a metal ion - usually zinc - and water in the active site).

11. Cysteine proteases use an ion of the sulfhydryl group of cysteine to act as a nucleophile to attached the carbonyl peptide bond and facilitate breakage of the peptide bond.

The magician would bid all adieu
Disappearing from audience view
At the end of the session
There was only one question
Was that just a stage he went through?

12. Aspartyl proteases employ two aspartic acid side chains to hold water in place and use an ion of one of them to act as a nucleophile to attack the peptide bond.

13. Metalloproteases use a metal ion to hold water in place so it can be ionized to act as a nucleophile to attack the peptide bond.

14. Carbonic anhydrase is an enzyme that catalyzes the joining of carbon dioxide and water to form carbonic acid.

15. A zinc ion (held in place by three histidines in the active site of carbonic anhydrase) plays an important role in the catalysis of the enzyme by binding a water molecule. A subsequent loss of a proton by water is necessary for catalysis. Notably, the enzyme has maximal activity at a high pH (where protons are easily removed) and a lower activity in an acidic pH (6.0).

16. The limiting step in the action of carbonic anhydrase is the abstraction of the proton from water. Buffers and/or bases help facilitate this and thus speed the reaction.

17. Restriction enzymes are bacterial enzymes that can cleave DNA by breaking phosphodiester bonds between adjacent nucleotides in the molecule.

18. Restriction enzymes act at specific nucleotide sequences. When these bind in the enzyme, it changes shape and this shape change causes a bend in the DNA. The bend provides a "pocket" for a water molecule and magnesium ion to be positioned properly so that the water can be activated to be a nucleophile to attack the phosphodiester bond between nucleotides to cleave it.

19. Restriction enzymes are paired with a methylase in bacterial cells. The methylase puts a methyl group on the same sequence the restriction enzyme would otherwise cut. When cellular DNA is protected in this way, the restriction enzyme cannot cut the cellular DNA, but it can cut invading viral DNA if it gets to it before the methylase does.

20. Restriction enzymes bind to DNA and "slide" along the double helix. When they reach the sequence they cut at (recognized by hydrogen bonds), the DNA is bent and a magnesium ion is allowed into the complex to facilitate the activation of water to nucleophilically attack the phosphodiester bond, cleaving it.

21. Myosin is a protein that undergoes a significant shape change upon hydrolysis of ATP. This action allows it to move along actin filaments and facilitates muscular contraction.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Allostery and Regulation

1. Aspartate transcarbamoylase (ATCas) is an enzyme that catalyzes the first step in pyrimidine biosynthesis (aspartate + carbamoyl phosphate <=> N-carbamoylaspartate). This enzyme is allosterically regulated in both a positive and negative fashion and also responds to the binding of the substrate (aspartate) to it.

I had asked to try out something new
On the burger I ordered from you
Perhaps you got flustered
When adding the mustard
Because it seems like Dijon Vu

2. CTP, the end product of pyrimidine biosynthesis, inhibits the enzyme, whereas ATP (a purine and an indicator of high energy) activates the enzyme. This phenomenon - where the end product of a metabolic pathway inhibits the first enzyme in the pathway - is known as feedback inhibition. Feedback inhibition is mediated allosterically - when a small molecule binds to a protein and affects the protein's activity.

3. ATCase has 12 subunits - 6 catalytic and 6 regulatory. The smaller regulatory subunits bind CTP, but not the catalytic subunits.

4. Binding of CTP to the regulatory subunits of ATCase causes the enzyme to stabilize (lock) in the T state (tight, less reactive state). In the T state, the quaternary structure of the enzyme the enzyme exhibits reduced affinity for substrate. In the opposite state, the R state (relaxed state, more reactive state), the enzyme has increased activity and a higher affinity for substrate. ATP stabilizes the R state of the enzyme.

5. In the absence of ATP and CTP, the enzyme can freely flip between the T and R states, but the T state tends to predominate.

6. If one removes all of the ATP and CTP from the enzyme and starts adding increasing amounts of the enzyme's normal substrate (aspartate) and then measures the reaction rate (velocity), one discovers a sort of sigmoidal plot, which indicates that the enzyme is actually changing as more aspartate is added to it. The actual change is a flip from the T state to the R state stabilized by the aspartate.

7. Students should be familiar with the effects of ATP, CTP and increasing aspartate on ATCase and where they bind on the enzyme to exert their effects.

8. A manmade artificial substrate, known as PALA, binds to ATCase and inhibits the enzyme, but interestingly locks it in the R state. Though the enzyme can't catalyze a reaction when bound to PALA, it is clear that its form changes, again indicating that enzymes are changed by the binding of substrate and that T and R states exist.

When the food color was misapplied
To the meat loaf the baker supplied
The patrons ingested
But barely protested
Even though they were dyeing inside

9. Protein kinase A is an enzyme involved in covalent modification of enzymes. Like all kinases, it catalyzes the addition of a phosphate to a molecule. Since it is a protein kinase, the molecules it attaches phosphate to are proteins.

10. Phosphates get attached to hydroxyl side chains in proteins. Protein kinase A attaches phosphate to serine or threonine. Other protein kinases can attach phosphate to tyrosine side chains.

11. Protein kinase A is controlled by allosteric means. It is composed of two regulatory subunits and two catalytic subunits. The overall complex is called R_2C_2. When the catalytic subunits are bound to the regulatory subunits, they cannot catalyze reactions. However, when the molecule cAMP binds to the regulatory subunits, the catalytic subunits are released and become active. When active, they covalently modify proteins by adding phosphates.

12. Zymogens are enzymes that are synthesized in an inactive form whose activation requires covalent modification, usually proteolytic cleavage. Examples include digestive enzymes, such as trypsin, chymotrypsin, elastase, and carboxypeptidase whose enzymatic activity might be harmful to the tissue where they are being made. Trypsin is the primary activator of an entire class of proteolytic enzymes. Improper activation of trypsin in or close to the pancreas can lead to pancreatitis, which arises when the proteases attack proteins in the pancreas.

13. Activation of chymotrypsinogen to chymotrypsin, for example, requires trypsin. Trypsin makes an initial cleavage between amino acids 15 and 16. A disulfide bond keeps the two pieces from coming completely apart, however. This creates an intermediately active form of chymotrypsin called pi-chymotrypsin. This form cleaves itself

to remove two dipeptides and this results in full chymotrypsin activity. The three polypeptide pieces are held together by disulfide bonds. Note that three very minor changes in the chymotrypsinogen zymogen have converted a completely inactive enzyme to a fully active one.

14. Alpha-one-antitrypsin is an inhibitor of many proteases, including trypsin and elastase. Normally alpha-one-antitrypsin binds to elastase and prevents it from damaging tissue. People deficient in the factor have proteins in their alveolar walls of the lung (and other connective tissue proteins) destroyed, ultimately resulting in emphysema. Smoke from cigarettes will react with HEALTHY alpha-one-antitrypsin, causing a methionine residue to be oxidized, which, in turn, prevents alpha-one-antitrypsin from binding to elastase, allowing elastase to damage alveolar tissue and eventually cause emphysema.

15. Blood clotting is a process that is also tightly controlled by zymogens. The process is a cascade of catalytic events in which one enzyme activates another, which in turn activates hundreds/thousands, which in turn activate millions. Cascades are great for amplifying a signal.

16. Two pathways activate the blood clotting process, the intrinsic pathway and the extrinsic pathway. Ultimately these cascades lead to the conversion of prothrombin (zymogen) to thrombin (active enzyme). Thrombin, in turn, converts fibrinogen (zymogen) to fibrin (mesh former as a result of polymerization).

17. Prothrombin must bind calcium in order to be held near the site of the wound to be activated. Binding of calcium by prothrombin allows it to anchor itself to the phospholipid membranes derived from blood platelets after injury. At this site, prothrombin can be readily converted to thrombin because at the site of the injury are other enzyme that can activate prothrombin to thrombin. To enable prothrombin to strongly bind calcium, glutamate residues in it must be carboxylated (addition of a carboxyl group). This reaction is catalyzed by an enzyme that uses Vitamin K as a cofactor. Compounds like coumarin or warfarin that block vitamin K sites on the enzyme act as "blood thinners", reducing the likelihood of blood clotting by inhibiting the carboxylation of prothrombin.

18. Removal of blood clots involves an enzyme called plasmin, which is synthesized as a zymogen called plasminogen. Plasminogen is converted to plasmin by tissue-type plasminogen activator (t-PA). t-PA can be extremely effective in initiating the cascade to dissolve the unwanted blood clot.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Carbohydrate Nomenclature, Structure and Function

1. Simple carbohydrates are monosaccharides, also called sugars. These include glucose, galactose, and mannose.

2. The suffix '-ose' is used to designate saccharides. The number-related prefixes 'tri', 'tetr', 'pent', 'hex', 'hept', and 'oct' are used to designate saccharides with 3,4,5,6,7, and 8 carbons, respectively.

3. Monosaccharides with an aldehyde group are called aldoses. Those with a ketone group are called ketoses.

4. Glyceraldehyde and dihydroxyacetone are the simplest saccharides we call carbohydrates.

5. Carbon can have as many as four different molecular groups attached to it. If this happens, the carbon is chiral (or asymmetric) and the groups can be arranged in two different ways. These arrangements are called stereoisomers.

6. The letters 'D' and 'L' are an older nomenclature system used to designate whether a particular stereoisomer rotated polarized light rightwards or leftwards, respectively. We use the convention today that the 'D' isomer corresponds to the stereoisomer in which the lowest asymmetric carbon from the top (closest to the bottom) is written on the right side of the molecule.

7. Most biological sugars are in the D configuration.

8. Stereoisomers, such as D-glyceraldehyde and L-glyceraldehyde, are mirror images of each other.

9. When sugars differ in stereoisomeric configuration, they are called diastereomers. When stereoisomers are mirror images of each other, they are called enantiomers. When they differ in configuration of only one

carbon, they are called epimers. When they differ only in the configuration of the anomeric carbon, they are called anomers.

10. Cyclization of monosaccharides leads typically to five member or six member rings. These are called furanoses (five member rings) and pyranoses (six member rings). Cyclization arises from formation of hemiacetals in aldoses and hemiketals in ketoses.

11. Haworth structures refer to the ring forms of sugars. The straight chain forms are referred to as a Fischer projections.

12. Cyclization creates a new asymmetric carbon. This carbon is called the anomeric carbon and it can exist in the alpha (down position) or beta (up position) configurations. If the hydroxyl group on the anomeric carbon is unaltered, the ring and linear forms of the sugar can reversibly form. Thus, a sugar in the beta configuration can, in solution, 'flip' to the alpha form by going to the linear form and then reverting back to the ring structure in the alpha configuration. If the hydroxyl of the anomeric carbon is altered (by methylation, for example), the linear structure cannot form and 'flipping' cannot occur.

13. Altering the hydroxyl group on the anomeric carbon results in creation of a glycoside. Glycosides are commonly created during formation of disaccharides and longer carbohydrates.

14. A given sugar such as beta-D-glucose can have different conformations that have different shapes. These are referred to as 'boat' and 'chair' forms. Chair forms are generally favored over boat forms due to less steric hindrance.

The story is out so they told it
Twas for sale but they just never sold it
It really is sad
That the business was bad
Online Origami has folded

15. Linking together of more than one sugar residues creates higher order saccharides. These include disaccharides (two sugars), trisaccharides (three sugars), oligosaccharides (several sugars), and polysaccharides (many sugars).

16. Most of the linkages in higher order saccharides involve glycosidic bonds.

17. Disaccharides include sucrose (glucose + fructose), lactose (glucose + galactose), and maltose (two glucoses).

18. Sucrose is a non-reducing sugar (has no free anomeric hydroxyl), whereas lactose is a reducing sugar (has a single free anomeric hydroxyl).

19. Oligosaccharides are components of glycoproteins.

20. The most common polysaccharides include glycogen (energy storage in animals), cellulose (structural integrity in plants), starch (energy storage in plants), chitin (exoskeleton of insects).

21. Polysaccharides can be homopolymers (contain only one sugar residue) or heteropolymers (contain more than one sugar residue). Homopolymers include glycogen (glucose in alpha 1-4 linkages plus extensive alpha 1-6 branches), cellulose (glucose in beta 1-4 linkages), amylose (glucose in alpha 1-4 linkages), amylopectin (glucose in alpha 1-4 linkages plus some alpha 1-6 branches), and chitin (N-acetyl-D-glucosamine in beta 1-4 linkages).

22. Glycogen is an animal energy storage polysaccharide, amylopectin and amylose combine to form starch, which is a plant energy storage polysaccharide, cellulose is a plant structural polysaccharide, and chitin is a component of insect exoskeletons.

23. The enzyme cellulase is required to digest the beta 1-4 bonds of cellulose. Most animals do not contain cellulase. Ruminants and ungulates contain the bacterium that makes that enzyme.

24. Glycosaminoglycans are polysaccharides that contain either N-acetylgalactosamine or N-acetylglucosamine as one of their monomeric units. They are polyanionic and have interesting chemical properties, as a result. Examples include chondroitin sulfates and keratan sulfates of connective tissue, dermatan sulfates, heparin, hyaluronic acid, and others.

25. Proteoglycans are complexes of proteins and glycosaminoglycans that form feathery structures.

26. Glycoproteins consist of a protein linked to an oligosaccharide, usually via an 'N' or an 'O' linkage. N linkages occur through asparagine of the protein. O linkages occur across serine or threonine of the protein.

On the latest test I was confused
By the math trigonometers use
It went rather fast
I hope that I passed
I don't have a secant to lose

27. Oligosaccharides on proteins (glycoproteins) and lipids (glycolipids) have functions in cellular identity and can be recognized and bound by immunoglobulins.

28. Oligosaccharides on the surface of cells help give them their identity. For example, A,B, and O blood group antigens give rise to the various blood types and these arise from carbohydrates on their cell surfaces.

29. Transplanted organs suffer rejection when the new organ has a different oligosaccharide pattern than the organ the recipient originally had. This encourages the immune system to attack it as foreign.

30. All N-linked glycoproteins have the same core of five carbohydrate residues. N-linked glycoproteins have glycosylation (addition of carbohydrate residues) occurring in the endoplamic reticulum and Golgi complex of the cell. O-linked glycoproteins have glycosylation occuring only in the Golgi complex.

31. Movement of modified proteins from the endoplasmic reticulum to the Golgi complex allows for additional carbohydrate modifications to occur, followed by targeting to 1) the cell membrane, 2) release from the cell, or 3) the lysosome.

In their racing, the two silk worm guys
Gave it all with an old college try
The results in the end
Did their future portend
Since they both ended up in a tie

32. Oligosaccharides destined to be linked to proteins to make glycoproteins are "built" on dolichol phosphate on the outer portion of the endoplasmic reticulum and then this "flips" to the inside for attachment.

33. Specific carbohydrate residues on the surface glycoproteins of blood cells are binding targets for hemagluttanin proteins on the surface of flu viruses. To enter a cell, the virus must cleave the sialic acid off with a neuraminidase enzyme (also on the flu virus's surface). Anti-flu drugs like tamiflu act by inhibiting the action of the neuraminidase.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Signaling

1. Signaling is essential for cells in multicellular organisms to communicate with each other.

2. A common signaling system is that of the beta-adrenergic receptor, which works as follows. A ligand, such as epinephrine (also called adrenalin) is released into the bloodstream in response to a stimulus. This molecule is called the first messenger. When it arrives at the target cell, it binds to the receptor, causing the receptor to change shape slightly. The result of this shape change is that on the inside of the cell, the receptor acts on a G protein (see below) to activate it. The activated G protein, in turn, activates the enzyme adenylate cyclase, which, in turn, begins to catalyze synthesis of cAMP. cAMP is a so-called second messenger, which acts by binding to Protein Kinase A (PKA) to activate it. The activated PKA begins phosphorylating a set of enzymes, turning them on or off (depending on the enzyme).

3. Phosphorylation of proteins known as transcription factors can activate or inactivate them. When activated they will turn on transcription of specific genes in the DNA.

4. Thus, signaling can have rapid effects (controlling enzyme activities) or slower effects (controlling gene expression by controlling transcription).

5. Receptors of the first messenger have similarity in structure, consisting of a polypeptide chain that spans the cell membrane 7 times. Such proteins are called 7TM proteins.

6. G proteins get their name from the fact that they bind guanine nucleotides (GDP and GTP). G protein complexes have three subunits - alpha, beta, gamma. Cells have 'families' of G

protein complexes arising from the fact that there are multiple, slightly different versions of the individual subunits in the genome and these can be paired in many ways. The alpha subunit binds to GDP or GTP. When the alpha subunit is bound to GDP, it also binds the beta and gamma subunits. The G protein complex is thus inactivated. When the beta-adrenergic receptor binds its ligand, the receptor stimulates the 'loading' of GTP onto the alpha subunit, displacing GDP in the process. Upon binding GTP, the alpha subunit dissociates from the beta and gamma units. The alpha subunit is then free to bind other target proteins and is thus 'active' when bound to GTP.

7. G proteins have an enzymatic activity that slowly breaks down GTP to GDP within the alpha subunit. This activity is very important because it ensures that the G protein will not be left in the 'on' state permanently. When the alpha subunit is bound with GTP, it can bind to adenylate cyclase (an enzyme embedded in the cell membrane) and activate it. When the alpha subunit has GDP, it is bound with the beta/gamma subunits and CANNOT bind to adenylate cyclase.

In the sea it can get quite complex
Tracking down the partic'lars of sex
If an 8-legged guy
Likes his mom on the sly
Should they call him an Octopus Rex?

8. Cells have two ways of turning off the beta adrenergic receptor. The first involves simple dissociation of the epinephrine ligand from the receptor. This leaves the receptor in the 'off' state. The second method involves phosphorylation of the receptor (by a receptor kinase). The phosphorylated receptor is then bound by the protein called beta-arrestin that binds the receptor and inhibits the activation of G proteins.

9. cAMP in cells acts on a kinase known as Protein Kinase A (PKA). PKA is composed of four subunits - two identical catalytic subunits (C) and two identical regulatory subunits (R). When the complex is present as C_2R_2, the PKA is inactive. Binding of cAMP to the R subunits causes them to dissociate from the C subunits. The freed C subunits are therefore active.

The philosophers think that it's great
The optometrist's zen mental state
There's no big surprise
It includes both the eyes
Get up early, work hard, and dilate

10. Signaling through the adrenergic receptor has the effect of increasing blood glucose. Insulin is a hormone that counters the effect of epinephrine. It should be noted that increasing concentration of cAMP in cells results in an increase of blood glucose. Phosphodiesterase breaks down cAMP. Inhibitors of phosphodiesterase, such as caffeine, have the effect of increasing blood glucose.

11. Other receptors involved in signal transduction (signaling) act in different ways. For example, some receptors stimulate the activity of the enzyme Phospholipase C. This enzyme acts on a

membranous molecule called phosphatidyl inositol (or PIP_2). Cleavage of PIP_2 by phospholipase C results in production of TWO second messengers. One of these, diacylglycerol (DAG) remains near or in the lipid bilayer where it stimulates another kinase, Protein Kinase C. Protein Kinase C acts to phosphorylate numerous proteins/enzymes to activate/inactivate them, depending upon the enzyme. The other second messenger produced by phospholipase C cleavage is inositol 1,4,5 triphosphate (also called IP_3). IP_3 is soluble in the cytoplasm and it acts there to stimulate the release of calcium from intracellular storage areas holding calcium.

12. Calcium may be thought of as a kind of 'third' messenger in the process of signaling. Cells normally must keep the concentration of the ion low so as to prevent it from binding to proteins and precipitating DNA. Calcium is essential for muscular contraction.

13. EF Hands are important structural domains of calcium binding proteins. Calmodulin is one such protein.

14. Calmodulin binds calcium, helping to keep its concentration relatively low. Upon binding calcium, calmodulin changes shape and this change in shape allows it to bind to other proteins that it wouldn't otherwise be able to bind to. One class of these are the CaM kinases that are stimulated to phosphorylate proteins when calmodulin binds.

15. Another signaling mechanism is mediated directly through receptors and doesn't involve G proteins. A good example is the insulin receptor. This receptor is normally present in the membrane of a target cell in a dimeric form and consists of two extracellular alpha subunits and two

intracellular beta subunits. The beta subunits contain the active site of the enzyme, a tyrosine kinase. In the absence of insulin binding, the subunits are non-phosphorylated and the kinase is inactive.

16. Binding of insulin by the insulin receptor moves the units of the dimer closer together. This, in turn, causes the two tyrosine kinase sites to phosphorylate tyrosine residues on the opposite one, activating it. The two kinases then phosphorylate tyrosines at other places on each beta subunit.

In their schools little fishies await
The subject they all think is great
They don't line in a path
To sign up for math
Instead all of them take debate

17. The phosphorylated tyrosines on the insulin receptor are binding targets for proteins known as insulin receptor substrates (IRS-1). The protein contains a common domain, called SH_2 that recognizes and binds phosphorylated tyrosines. Many proteins have SH_2 domains.

18. When IRS-1 binds to the insulin receptor, it gets phosphorylated on tyrosines, as well, and these phosphorylated tyrosines are bound by the SH_2 domain of an enzyme known as phosphatidylinositide-3-kinase. When phosphatidylinositide-3-kinase binds to the IRS protein, it is stimulated to phosphorylate PIP_2 in the membranes, making PIP_3.

19. PIP_3 in the membrane is the binding target for PDK1 (PIP_3-dependent protein kinase). PDK1 is thus activated in this way and it phosphorylates another kinase known as Akt. Akt is NOT membrane bound and upon phosphorylation is activated. It can then move through the cell, phosphorylating other proteins and activating pathways. One of the pathways it activates results in the movement of the protein known as GLUT4 to the cell surface. GLUT4 is a glucose transport protein that transports glucose into cells.

20. Another signaling system is that going through the epidermal growth factor (EGF) receptor. Epidermal growth factor is a small polypeptide whose action stimulates cells to divide and grow. The EGF receptor that it binds to exists in cell membranes as a MONOMER. Binding of EGF to the monomeric receptor causes the receptor to dimerize.

My dog has received a bad mark
On his driving test out near the park
He drove with both paws
And obeyed all the laws
But just couldn't parallel bark

21. The EGF receptor, like the insulin receptor, has an intracellular domain that is a tyrosine kinase. Dimerization of the receptor causes the carboxy-terminal portion of the receptor to become phosphorylated at tyrosines.

22. Genes in which mutations can happen that lead to uncontrollable growth are known as oncogenes. The

unmutated forms of these genes perform important signaling or control functions. These unmutated genes are known as proto-oncogenes.

23. Ras is a proto-oncogene. Mutations of ras that interfere with its ability to cleave GTP to GDPcan lead to uncontrolled cellular growth.

24. One type of leukemia (CML) arises from DNA rearrangement that joins together two gene-coding sequences known as BCR and ABL. ABL is a tyrosine kinase that plays an important role in controlling when cells divide. When BCR is joined to ABL (making BCR-ABL), the fusion that arises is made in greater quantities than ABL itself and it still contains the tyrosine kinase activity. Thus, the overexpressed fusion stimulates cells to divide uncontrollably.

25. BCR-ABL tumors are successfully treated with a tyrosine kinase inhibitor known as Gleevec.

The sailor is singing with ease
On his ship in a rather strong breeze
For his kind of a song
He can draw a big throng
Every time that he hits the high seas

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Metabolic Control

1. Metabolism is literally the chemistry of life. Schematic diagrams of metabolism are the equivalent of highway maps for a city. Central pathways, like glycolysis, are the equivalent of the main streets.

2. To understand chemical reactions in cells, we must understand the thermodynamics (energy) of the reactions.

3. The free energy of a process is the energy available to do useful work. The free energy of a process is called the Gibbs Free Energy.

4. Most commonly, we are concerned with the change in free energy for a system. For example, for an enzymatically catalyzed reaction, we are interested in the change in free energy between the reactants and the products. The change in free energy (ΔG) indicates the favorability of a process.

    a. If the ΔG for a process is negative, the process is favored.

    b. If the ΔG for a process is positive, the process is not favored (in fact, the reverse of the process is favored).

    c. If the ΔG for a process is zero, the process is at equilibrium.

Mister Bach was a wonderful bloke
Making music that oft would evoke
A satisfied smile
Dramatic in style
Thank goodness he went for baroque

Example: For A <=> B,

if ΔG is negative, A->B is favored.

if ΔG is positive, B-> A is favored.

if ΔG is 0, B-> A and A-> B are equally favored. The system is at equilibrium.

5. The chemical potential of a component A is equal to the chemical potential at the standard state plus RT ln[A]. For a reaction of multiple components

aA + bB <=> cC + dD

6. ΔG° is the ΔG measured under standard conditions (all products and reactants at 1M). Here, $\Delta G = \Delta G^\circ$

7. For biological systems, we define a $\Delta G^{\circ\prime}$ (note the prime at the end) to define standard conditions for aqueous solutions at pH 7.0.

A violation of Sir Isaac was found
By young Megan on her way to the ground
She’s not in Smithereens
Because on trampolines
What goes down must go up then go down

8. ATP is a source of energy in cells because the ΔG of the hydrolysis reaction is very negative (releases much free energy). Hydrolysis of ATP directly to yield energy (like burning of wood to heat a house) is not the mechanism used by cells to drive reactions. Instead, hydrolysis of ATP is coupled to energetically unfavorable reactions to make them proceed.

9. Cells must have ATP to accomplish work (muscular action), transmit information (nerve signals), signal each other (cellular signaling), and synthesize important biochemicals.

10. Oxidation is used to provide the energy necessary to make ATP. ATP energy is used to provide reduction necessary to biosynthesize compounds like fats and fatty acids.

11. The oxidation state of a molecule is related to its available energy. The higher the oxidation state of a molecule, the less energy that can be obtained from it. Thus, glucose, which has a higher oxidation state than fatty acids, provides less energy to cells than fatty acids.

12. ATP is made from ADP by phosphorylation. There are three types of cellular phosphorylation in nature. Substrate level phosphorylation

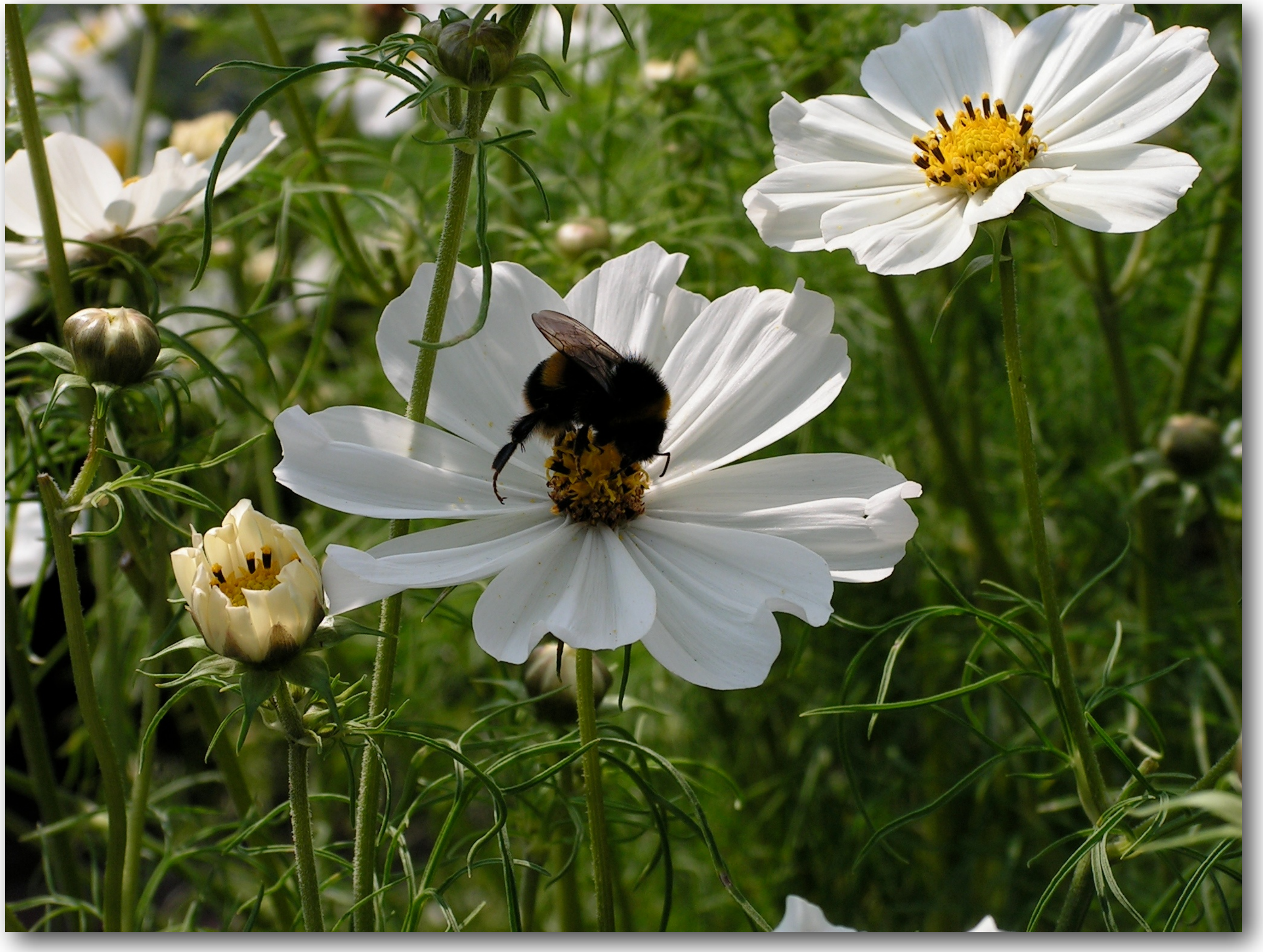
occurs when high energy phosphate molecules (like creatine phosphate) transfer their phosphate directly to ADP to form ATP. Oxidative phosphorylation occurs as a result of actions in the mitochondria, ultimately caused by oxidation. Photophosphorylation (occurs only in photosynthetic organisms) uses light energy to make ATP.

13. Creatine phosphate is a backup ATP source for muscle cells. This arises from the following reaction

Creatine + ATP <=> Creatine phosphate + ADP

($\Delta G^{\circ\prime} = +\ 12$ kJ/mol)

14. Normally when at rest, this reaction moves to the RIGHT, due to high ATP concentrations. Upon exercising, however, ATP concentrations drop, and the reaction moves to the left, restoring ATP levels, at least temporarily.



15. For every oxidation (loss of electrons) by one molecule, there is a reduction (gain of electrons) by another one. In biological systems, electron carriers, such as NAD^+/NADH and FAD/$FADH_2$ are electron carriers. When a biological molecule is oxidized, electrons are given (in pairs) to either NAD^+ or FAD to form NADH or $FADH_2$. NADH and $FADH_2$ can also donate electrons to biological molecules, thus reducing the biological molecules.

16. An example oxidation/reduction reaction that might occur in cells is

Alcohol + NAD^+ <=> Aldehyde/Ketone + NADH + H^+

17. Catabolism generally involves oxidation and/or breakdown of large molecules (proteins, fats, carbohydrates) into smaller ones (many of these converge at acetyl-CoA). Catabolism releases energy that is used to make ATP. Anabolism generally

involves reduction and/or synthesis of large molecules from small ones. Anabolism requires an energy source - often from ATP.

18. ATP energy in anabolism is used to drive energetically unfavorable reactions by coupling the hydrolysis of ATP (an energetically favorable process) with the reaction that is energetically unfavored (such as the addition of phosphate to glucose, as noted in class). By doing this coupling, an unfavorable reaction becomes energetically favorable. Thus, the synthesis of large molecules from small ones is not energetically favorable, but when energy from ATP is provided, they become energetically favorable.

19. Besides ATP energy (or GTP or UTP or CTP, as appropriate), other mechanisms available to cells to "drive" reactions forward are to alter the concentrations of reactants and products. We define the phenomenon of "pushing" a reaction as increasing the amount of reactants. This has the effect of reducing the ΔG value and making a reaction more favorable in the forward direction. We also define the phenomenon of "pulling" a reaction as decreasing the amount of product. This too has the effect of reducing the value of ΔG and favoring a reaction to go forward.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Glycolysis & Gluconeogenesis

1. Glycolysis, the breakdown of glucose, is a catabolic pathway involving oxidation and yields ATP and NADH energy. Gluconeogenesis, the synthesis of glucose, is an anabolic pathway that involves reduction and uses ATP and NADH. There are 10 reactions in glycolysis. Students should know structures of fructose and glucose compounds, all enzyme names, all molecule names, and reactions where the ΔG°' is strongly positive, or strongly negative.

2. Note that glycolysis has two phases - an energy investment phase requiring input of ATP energy and an energy realization phase where ATP is made.

3. In reaction #1 of glycolysis, hexokinase catalyzes transfer of phosphate to glucose from ATP, forming glucose-6-phosphate, G6P. Thus, this step uses ATP, which provides the energy necessary for the reaction to proceed. It is an example of an energy-coupled reaction and the ΔG°' is strongly negative, thanks to the ATP hydrolysis.

4. Hexokinase changes shape as it binds to glucose. This property is consistent with that of an induced fit of an enzyme in the process of catalysis.

5. Reaction #2 of glycolysis is catalyzed by phosphoglucoisomerase. In it, G6P is converted to F6P. The ΔG°' for the reaction is close to zero.

6. Reaction #3 is the primary regulatory reaction of glycolysis. It is catalyzed by phosphofructokinase (PFK). Note that this reaction also requires ATP. PFK is the most important regulatory enzyme for glycolysis. The molecule made in the process, F1,6BP, is a high energy molecule and the

At the bookstore I asked timidly
For some help to get phobia-free
The clerk pointed to
A brand new review
But I fear that it may not help me

energy in the molecule is needed in the next reaction. The reaction is another example of an energy-coupled reaction and the $\Delta G^{\circ\prime}$ is strongly negative, thanks to the ATP hydrolysis.

7. Reaction #4 is catalyzed by aldolase. It has a strongly positive $\Delta G^{\circ\prime}$. In the cell, however, the reaction is pulled by reactions ahead of it (which remove products) and pushed by reactions behind it (which increase amounts of reactants), making the ΔG favorable (negative). The products of this reaction are G3P and DHAP.

8. The energy barrier of reaction 4 is overcome by 'pushing' (increasing concentration of reactatnts) and 'pulling' (decreasing concentration of products) of the reaction.

9. Reaction #5 is catalyzed by the 'perfect' enzyme known as triose phosphate isomerase. The reason the enzyme operates so fast is to prevent accumulation of a toxic intermediate. The product of the reaction is G3P. The $\Delta G^{\circ\prime}$ is close to zero, so this reaction is readily reversible. Everything after this step has two molecules of each.

10. Reaction 6 is the only oxidation in glycolysis. It is catalyzed by glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The energy of the oxidation is used to put phosphate onto the acid produced by the oxidation. The products are NADH and 1,3 BPG. The latter has high energy (higher than ATP).

11. Reaction #7 is catalyzed by phosphoglycerate kinase and it includes a substrate level phosphorylation (transfer of a phosphate from a molecule directly to ADP) to make ATP.

12. Reaction #8 is catalyzed by phosphoglycerate mutase and it simply involves rearrangement of the 3PG into 2PG. The ΔG°' is close to zero and the direction of the reaction is driven by cellular concentrations. Note that the mutase creates as an intermediate 2,3BPG, which is the molecule hemoglobin bound to and caused it to release oxygen.

13. Reaction #9 is catalyzed by enolase and involves removal of water from 2PG to form PEP, which is a highly energetic compound.

14. Reaction #10 is the "Big Bang" of glycolysis. It is catalyzed by the enzyme pyruvate kinase and in the reaction, a substrate level phosphorylation yields ATP. Note that the ΔG°' is very strongly negative, helping to pull all the reactions preceding it to a large extent. The enzyme is allosterically inactivated by ATP and allosterically activated by F1,6BP. The latter activation is an example of "feed forward" activation. Pyruvate kinase is also inactivated by phosphorylation, as will be seen in glycogen metabolism.

15. The phenomenon of redox balancing is important for glycolysis. Redox balancing relates to the relative amount of NAD^+ and NADH in the cell. Remember that reaction 6 is very sensitive to the ratio of NAD^+/NADH.

16. Pyruvate has three separate fates, depending on conditions and the cell type. When oxygen is present, there is plenty of NAD^+, so aerobic cells convert pyruvate to acetyl-CoA for oxidation in the citric acid cycle. When oxygen is absent, NAD^+ levels can go down, so to prevent that from happening, pyruvate is converted to either lactate (animals) or ethanol (bacteria/yeast). Either of these last two conversions requires NADH and produces NAD^+. NAD^+ made in this way can be used to keep reaction 6 of glycolysis going.

17. Anaerobic conversion of NADH to NAD^+ provides much less ATP energy to cells than when oxygen is present. Anaerobic metabolism of glucose generates only 2 ATPs per glucose,

whereas aerobic metabolism of glucose generates 38 ATPs per glucose.

The Asian chef worked with aplomb
For a name for his restaurant’s dot com
He got most creative
Sticking close to the native
And dubbed it the Viet Nom Nom

18. Other sugars than glucose can be metabolized by glycolysis, if they are converted to intermediates of glycolysis.

19. Entry of fructose to the glycolysis cycle may be problematic in some cases. Fructose can be converted to F6P by hexokinase. Fructose can also be converted to fructose-1-phosphate (F1P) by fructokinase. Conversion of F1P to glyceraldehyde and DHAP allows fructose to be metabolized by glycolysis without being controlled by PFK. Ingestion of a lot of fructose (via high fructose corn syrup in many foods) may be a factor in obesity.

20. Other sugars than glucose can be metabolized by glycolysis, if they are converted to intermediates of glycolysis.

21. Galactose can enter glycolysis by being converted to galactose-1phosphate followed by conversion (ultimately) to glucose-1-phosphate and subsequently to glucose-6-phosphate, which is a glycolysis intermediate.

22. Deficiency of galactose conversion enzymes results in accumulation of galactose (from breakdown of lactose). Excess galactose is converted to galactitol (a sugar alcohol). Galactitol in the human eye lens causes it to absorb water and this may be a factor in formation of cataracts.

23. Deficiency of the enzyme lactase leads to lactose intolerance

24. Regulation of glycolysis is controlled by three enzymes - hexokinase, PFK, and pyruvate kinase. Hexokinase's regulation is a bit complicated and is controlled partly by availability of substrate.

25. PFK is very unusual in being negatively regulated by a molecule (ATP) that is also a substrate. This is possible because the enzyme has an allosteric binding site for ATP in addition to the substrate binding site and the K_m for the allosteric site is higher than the substrate binding site.

26. Pyruvate kinase is regulated both allosterically and by covalent modification (phosphorylation/dephosphorylation). Phosphorylation of the enzyme by a protein kinase turns the enzyme activity down, whereas F1,6BP acts as an allosteric activator. This activation is known as feedforward activation.

27. Feedforward activation is rare in metabolism. It is a term used to describe a metabolic product (such as F1,6BP above) that ACTIVATES an enzyme that catalyzes a reaction further ahead of it in a

If his hair was not tightly held on
Captain Kirk’s message might be withdrawn
And replaced with a thought
That would not be forgot
Baldly going where no man has gone

metabolic pathway. In glycolysis, feedforward activation acts to start the pyruvate kinase reaction and PULLs the reactions forward to get over the energy "hump" of the aldolase reaction.

28. Hypoxia refers to the condition where cells are short of oxygen. Since oxygen is necessary for maximum energy production from glucose, they must respond to this condition. One way they respond is by making a transcription factor known as Hypoxia Induction Factor 1 (HIF-1). HIF-1 activates transcription of genes involved in glucose transport and glycolysis. Cancer cells are frequently hypoxic and induce HIF-1, as well. Another way cancer cells battle hypoxia is to stimulate the growth of blood vessels to them by making another factor known as angiogenin. Blocking HIF-1 and angiogenin are anti-cancer therapies.

The cable guy felt quite impelled
In the wedding his wife and he held
To get everything right
He would work overnight
Making sure the reception excelled

29. Glycerol is a breakdown product of fat metabolism. Glycerol can be metabolized in glycolysis by conversion to glycerol-3-phosphate and then to DHAP, a glycolytic intermediate.

30. Gluconeogenesis accomplishes the reverse of glycolysis - synthesis of glucose from pyruvate using four different enzymes to replace three energetically unfavorable reactions in glycolysis.

31. Gluconeogenesis does not occur in all tissues of the body. The primary gluconeogenic organs of the body are the liver and part of the kidney.

32. The enzymes unique to gluconeogenesis are Pyruvate Carboxylase and PEP carboxykinase (PEPCK) instead of Pyruvate Kinase of glycolysis, Fructose 1,6 Bisphosphatase (F1,6BPase) instead of Phosphofructokinase (PFK) from glycolysis, and Glucose-6-phosphatase (G6Pase) instead of Hexokinase from glycolysis.

In the forest you should be aware
Flexibility’s really quite rare
You’d be surprised catching
Any wildlife out stretching
Except for a few yoga bears

33. F1,6BPase and G6Pase act by similar mechanisms, clipping a phosphate from their substrates and thus avoiding synthesis of ATP, which is what would be required if the glycolysis reactions were simply reversed.

34. One reaction of gluconeogenesis occurs in the mitochondrion. It is catalyzed by pyruvate carboxylase and yields the four carbon intermediate, oxaloacetate. The carboxyl group is added in forming oxaloacetate thanks to the coenzyme biotin, which carries carbon dioxide for attachment. The remaining reactions all occur in the cytoplasm, except for the G6Pase reaction, which occurs in the lumen of the endoplasmic reticulum.

35. Anabolic and catabolic pathways occurring at the same time and place create a futile cycle. Futile cycles generate heat, but that is the only product they make. Consequently, cells usually set up controls that turn one off when the other is turned on. If the same molecule has opposite effects on catabolic and anabolic pathways, the molecule is a reciprocal regulator of the pathways. Reciprocal regulation of catabolic and anabolic pathways is a very efficient means of control.

36. The Cori Cycle is a cycle of the body where the lactate of working muscles is dumped into the bloodstream. It travels to the liver where it is converted to pyruvate and used to make glucose in gluconeogenesis. The glucose is then dumped into the bloodstream, where it travels back to the muscles that need it.

On the most recent summer vacation
Only one place deserved condemnation
Its dry summer breezes
Gave us too many sneezes
When we got to our first dusty nation

37. Anabolic and catabolic pathways occurring at the same time and place create a futile cycle. Futile cycles generate heat, but that is the only product they make. Consequently, cells usually set up controls that turn one off when the other is turned on. If the same molecule has opposite effects on catabolic and anabolic pathways, the molecule is a reciprocal regulator of the pathways. Reciprocal regulation of catabolic and anabolic pathways is a very efficient means of control.

38. The enzymes of glycolysis that are regulated have corresponding gluconeogenesis enzymes that are also regulated. PFK and F1,6BPase exhibit the most complicated regulation. Both are controlled by several mechanisms. The most important one is the allosteric reciprocal regulation by fructose-2,6-bisphosphate (F2,6BP). F2,6BP activates PFK and inhibits F1,6BPase.

39. F2,6BP is made and degraded by two different portions of the same protein (We'll use PFK2 to refer to the kinase portion and FBPase-2 to refer to the phosphatase portion). The portion of the

PFK2 catalyzing the synthesis of F2,6BP from F6P is PFK2. The portion of the protein catalyzing the breakdown of F2,6BP to F6P is FBPase-2. The two activities are regulated by phosphorylation of the PFK2/FBPase-2 protein by protein kinase A. When phosphorylated, the PFK2 part of the enzyme is inactive and the FBPase-2 is active. When the phosphate is removed from the protein by phosphoprotein phosphatase, the PFK2 becomes active and the FBPase-2 becomes inactive.

40. Phosphorylation of the enzyme by protein kinase A is favored by 7TM signaling whereas dephosphorylation by phosphoprotein phosphatase is activated by signaling by insulin.

41. Thus phosphorylation of the PFK2 favors the breakdown of F2,6BP and the activation of gluconeogenesis and deactivation of glycolysis. Dephosphorylation of PFK2 favors the synthesis of F2,6BP and the activation of glycolysis and the deactivation of gluconeogenesis. This is the heart of reciprocal regulation of these pathways.

42. Pyruvate kinase, pyruvate carboxylase, and PEPCK are all regulated, as well. Pyruvate kinase is activated by feedforward activation by F1,6BP and is inhibited by ATP and alanine (a product easily made from pyruvate). Pyruvate kinase is also controlled by covalent modification as described in the previous highlights. Phosphorylation of the enzyme makes it less active, whereas dephosphorylation make it more active.

Jump to Chapter

1 / 2 / 3/ 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Glycogen Metabolism

1. The structure of glycogen consists of units of glucose linked in the alpha1-4 configuration with branches linked in the alpha1-6 configuration.

2. Glycogen differs from amylopectin in the amount of branching (glycogen has much more).

3. Glycogen is a storage form of energy that can yield ATP very quickly, because glucose-1-phosphate can be released very quickly.

4. Students should know the function/activities of the enzymes in glycogen breakdown - glycogen phosphorylase, phosphoglucomutase, and debranching enzyme.

5. Glycogen phosphorylase action on glycogen yields glucose-1-phosphate. Glycogen phosphorylase exists in two forms - phosphorylase a and phosphorylase b. Phosphorylase a differs from phosphorylase b only in that phosphorylase a contains two phosphates and phosphorylase b contains none. Phosphate is added to glycogen phosphorylase by the enzyme phosphorylase kinase.

6. Glucose-6-phosphate (G6P) has many different fates and sources. First, breakdown of glycogen produces G1P, which is readily converted to G6P. G6P can then go three different directions. In muscle and brain (and most other tissues), G6P enters glycolysis. In liver only, G6P enters gluconeogenesis and is converted to glucose for export to the bloodstream. In other tissues, G6P enters the pentose phosphate pathway and is oxidized to produce NADPH.

7. Breakdown of glycogen by glycogen phosphorylase involves phosphorolysis (use of a phosphate to cleave molecules) instead of hydrolysis. The advantage of this is that the energy of the alpha1-4 bond is used to add phosphate to glucose (forming G1P) instead of using a triphosphate to do so. This saves energy for cells.

The ocular surgeon can please
All the ladies with his surgeries
Though efficient at working
He is quite slow at flirting
That uncrossing eyes dodding tease

8. Glycogen phosphorylase catalyses phosphorolysis of glycogen to within 4 residues of a branch point and then stops. Further metabolism of glycogen requires action of debranching enzyme. Debranching enzyme removes three of the remaining four glucoses at a branch point and transfers them to another chain in an alpha1-4 configuration. The remaining glucose in the alpha1-6 configuration at the branch point is cleaved in a hydrolysis reaction to yield free glucose. It is the only free glucose released in glycogen metabolism.

9. Phosphoglucomutase interconverts G1P and G6P via a G1,6BP intermediate. The reaction is readily reversible ($\Delta G°'$ near zero) and the direction of the reaction depends on the concentration of substrates.

The camping group at the Great Lakes
Think their planners are really big flakes
The cause of dissent?
They can’t pitch a tent
Because of too many missed stakes

10. Synthesis of glycogen is not the simple reversal of the steps in glycogen breakdown. There is an energy barrier that must be overcome - synthesis of the alpha1,4 bond between adjacent glucoses in glycogen. This is accomplished by a 'side-step' reaction that creates uridine diphosphate glucose (UDP-Glucose or UDPG) from G1P.

11. Synthesis of UDPG requires UTP and G1P and produces UDPG and pyrophosphate (PPi). UDPG is made by action of the enzyme UDPG pyrophosphorylase.

(In the notes below, we refer to Glycogen Phosphorylase a as GPa and Glycogen Phosphorylase b as GPb. We also refer to Glycogen Synthase a as GSa and Glycogen Synthase b as GSb.

12. Glycogen phosphorylase is present in two covalently different forms, GPa and GPb. They differ in phosphorylation. GPa is phosphorylated and GPb is not.

13. GPb is converted into GPa by phosphorylation at two sites. Covalent modifications are DIFFERENT from allosteric controls, which interconvert the R and T states of BOTH GPa and GPb.

14. The different glycogen phosphorylase forms have different allosteric regulation, as well. GPb is converted to the R state by AMP and converted to the T state by ATP and G6P. The latter two are more commonly abundant in the cell than AMP, so we think of GPb as less active. GPa is converted to the T state by glucose (rarely present much). When glucose is

absent, GPa is automatically in the R state. Thus, we think of GPa as the more active form.

15. Phosphorylation of GPb to make GPa is catalyzed by an enzyme known as phosphorylase kinase. Full activation of phosphorylase kinase requires both calcium ions and phosphorylation by protein kinase A

16. Binding of epinephrine to the cell surface stimulates the following events in muscle relating to glycogen breakdown

    A. Epinephrine binds receptor
    B. Receptor activates a G protein to bind GTP
    C. Alpha subunit of G protein activates adenylate cyclase
    D. Adenylate cyclase catalyzes formation of cAMP
    E. cAMP activates protein kinase A
    F. Protein kinase A phosphorylates phosphorylase kinase, activating it.
    G. Phosphorylase kinase phosphorylates GPb, converting it to GPa
    H. GPa breaks down glycogen to yield G1P

17. Glycogen synthase (catalyzes formation of glycogen) exists in two different covalent forms - GSa (has no phosphate and is the most active) and GSb (has phosphate and is the least active).

18. Binding of epinephrine or glucagon to a cell surface receptor ultimately results in the phosphorylation of GPb and GSa to make GPa and GSb, respectively. The products of these phosphorylations - GPa and GSb have opposite activities. GPa is MORE ACTIVE and GSb is LESS ACTIVE. Thus, reciprocal regulation of glycogen metabolism is mediated through phosphorylation and dephosphorylation. The conversion of R and T states occurs allosterically.

19. Reversal of the process (removal of the phosphates of all of the phosphorylated enzymes) occurs as the

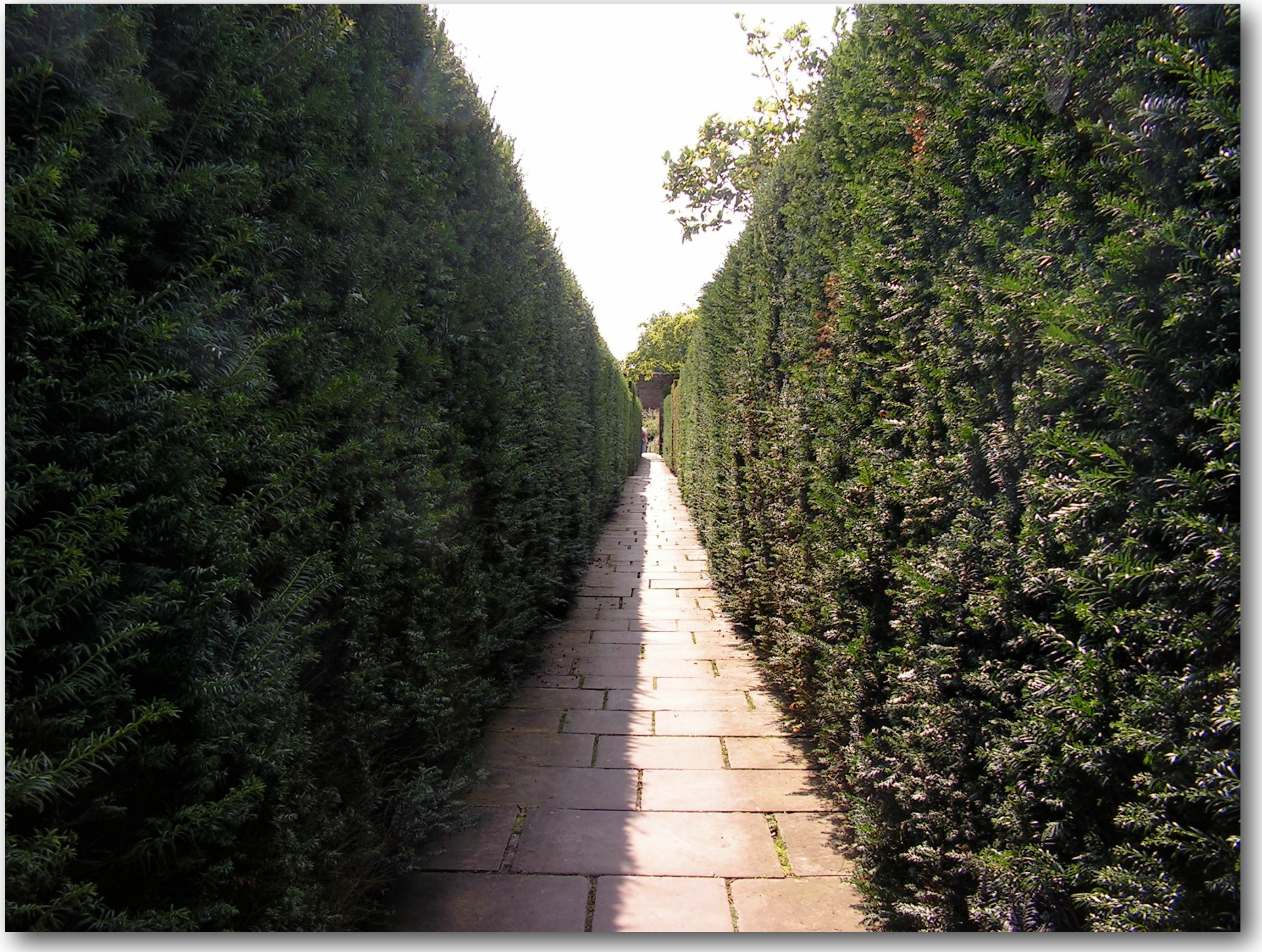
I went off to my grocer to beg
Lest I drop cooking standards a peg
The source of the fuss?
The high price of nuts
They are costing an almond a leg

result of catalysis by phosphoprotein phosphatase (PP-1). PP-1 is stimulated to be active by insulin. Thus, increasing glucose concentration in the bloodstream causes insulin to be released, which causes cells to take up glucose and also (inside the cell) to cause the glucose to be made into glycogen.

20. Reversing phosphorylations causes glycogen breakdown to cease and glycogen synthesis to begin. Remember that insulin is released in response to an increase in blood sugar and it stimulates cells to take up glucose. Thus, when the cells take up glucose, the glycogen synthesis system is stimulated to put it into glycogen and this occurs because insulin stimulates the activity of Protein Phosphatase (PP1), which is capable of removing phosphates from all the proteins described above.

21. So, when insulin binds the cell surface receptor, glycogen synthesis is stimulated (glycogen synthase is converted from the 'b' form to the 'a' form) and glycogen breakdown is inhibited (glycogen phosphorylase is converted from the 'a' form to the 'b' form). In addition, PP1 removes the phosphate from glycogen phosphorylase kinase, which stops it from phosphorylating additional glycogen phosphorylase enzymes.

22. Insulin is capable (via binding to a cell surface receptor) of reversing the action of the phosphorylation system. It does this by stimulating the activity of Protein Phosphatase I (PP1). This, in turn, causes ALL OF THE EARLIER PHOSPHORYLATIONS TO BE REVERSED.



23. By contrast, the protein kinase A phosphorylation system simultaneously activates glycogen breakdown (by making GPa) and inhibits glycogen synthesis (by making GSb), it also INACTIVATES the enzyme that removes phosphates (PP1).

The troubled young sprinter did vow
She'd make an adjustment somehow
Though the hurdles she feared
All were nonetheless cleared
It's good that she's over them now

24. PP-1 binds to a protein called G_M (in muscle) or G_L (in liver). When bound to G_L, PP-1 is held close to the glycogen phosphorylase, which is useful because this allows easy access to dephosphorylate it and turn it off.

25. Activation of PKA by epinephrine or glucagon causes G_M to be phosphorylated, which, in turn, causes PP-1 to be released in a less active form. PKA also phosphorylates the PP-1 inhibitor, which then binds PP-1 and inactivates it. Thus, the phosphorylation system shuts down the dephosphorylation system and vice versa, depending on which hormone has bound to the cell surface receptor.

26. GPa normally binds PP-1-G_L tightly and acts as a glucose sensor in liver cells. PP-1 is inactive when bound to GPa if GPa is in the R state. Increasing glucose concentration causes GPa to flip into the T state. When GPa is in the T state, PP-1-G_L is released from GPa, becomes active, and dephosphorylates GPa, forming GPb. Freed from GPa, can then PP-1 dephosphorylate GSb, forming GSa. As a result, glycogen synthesis is NOT activated until glycogen breakdown is first stopped.

27. Thus, addition of glucose to purified GPa and GSb causes conversion of GPa to GPb and GSb to GSa. Addition of glucose causes GPa to flip into the T state, which causes it to release PP-1-G_L to begin dephosphorylation of the two enzymes.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Citric Acid Cycle

1. Both oxidative decarboxylation (in higher cells) and non-oxidative decarboxylation (in yeast) use an enzymatic activity called the pyruvate dehydrogenase complex to convert pyruvate from glycolysis into acetyl-Coa for the citric acid cycle. This enzyme complex is in the mitochondrion and requires that pyruvate from the cytoplasm be transported to the mitochondrion. This complex includes the following:

    a. Pyruvate decarboxylase (some books call it "Pyruvate Dehydrogenase Component" (E_1)

    b. Dihyrolipoamide transacetylase (E_2)

    c. Dihyrolipoamide dehydrogenase (E_3)

2. It also uses five coenzymes, Thiamine Pyrophosphate (TPP), Lipoamide, NAD^+, FAD, and Coenzyme A (also called CoASH or CoA).

3. The mechanism of the reaction catalyzed by the complex is very similar to that catalyzed by the alpha-keto-glutarate dehydrogenase complex of the citric acid cycle. Both involve oxidation of alpha-keto acids.

4. In aerobic higher organisms, the reaction mechanism involves binding of pyruvate by an ionized TPP, decarboxylation, transfer to the lipoamide molecules, linkage of the acetyl group to CoASH to form acetyl-CoA, transfer of the electrons from the oxidation to FAD (forming $FADH_2$) and transfer of electrons from $FADH_2$ to NAD^+ to form NADH.

The Shakespearean critic was shrill
With the language he used to fulfill
His role as complainer
And major disdainer
That man was a fier at Will

5.In yeast fermentation, the reaction that occurs stops at the decarboxylation step with resolution to form acetealdehyde without loss/gain of electrons (no oxidation/reduction). Thus, enzyme activities E_2 and E_3 above are not needed in yeast fermentation. Acetaldehye in yeast fermentation is converted to ethanol by reduction, using electrons from NADH. Note that when oxygen is present, fermentation in yeast does not occur and activities E_2 and E_3 catalyze reactions just like animal cells, producing acetyl-CoA.

6. Mitochondria are the "power plants" of the cell and are the places where much oxidation occurs.

7. The citric acid cycle occurs in the mitochondrial matrix (inner portion of mitochondrion) and is found in almost every cell. In the cycle, two carbons are added from acetyl-CoA and two carbons are released as carbon dioxide.

8. Biological oxidation of intermediates in the citric acid cycle involve NAD^+ (reduced to NADH) and FAD (reduced to $FADH_2$). In the citric acid cycle, three NADH and one $FADH_2$ are produced, along with one high energy phosphate (GTP in animals, ATP in plants and bacteria) per acetyl-CoA that enters the cycle (Remember that one molecule of glucose yields two acetyl-CoAs for the cycle).

9. The citric acid cycle consists of two main parts - release of CO_2 (first part) and conversion to oxaloacetate (second part).

10. In the "first" reaction of the citric acid cycle, citrate synthase catalyzes the joining of the acetyl group from acetyl-CoA to oxaloacetate to make citrate. This reaction is VERY energetically favorable, due to breaking of the thioester bond in acetyl-CoA. The energetically favorable reaction helps to "pull" the relatively unfavorable reaction preceding it.

11. Aconitase catalyzes the rearrangement of citrate to isocitrate. Aconitase is inhibited by fluorocitrate. Fluoroacetate is a poison that can be used by citrate synthase to make fluorocitrate.

12. The first decarboxylation of the citric acid cycle is catalyzed by isocitrate dehydrogenase and the reaction is strongly favored to the right. The products of this reaction are NADH and alpha ketoglutarate.

13. Alpha ketoglutarate is an important intermediate for its involvement in anaplerotic reactions related to transamination. The mechanism of the enzyme acting on alpha ketoglutarate (alpha ketoglutarate dehydrogenase complex) is virtually identical to the mechanism of action of the pyruvate dehydrogenase complex and

My love for the stuff is insane
It’s addicting almost like cocaine
I guess I’m a softie
For freshly shipped toffee
That arrives in a big chew chew train

involves all of the same coenzymes. The products of this reaction are succinyl-CoA and NADH.

14. The only substrate level phosphorylation in the citric acid cycle is catalyzed by succinyl-CoA synthetase. The products of this reaction in the citric acid cycle are GTP and succinate. Note that the enzyme is named for the reverse reaction.

15. Succinate dehydrogenase contains a covalently-linked FAD electron carrier. The ΔG°' of zero allows the reaction to be readily reversed to produce succinate, when needed. The products of this reaction in the forward direction of the citric acid cycle are $FADH_2$ and fumarate (*trans* double bond). This reaction is similar to the first oxidation reaction for a fatty acid.

16. Addition of water to fumarate (catalyzed by fumarase) yields L-malate.

17. Oxidation of L-malate by malate dehydrogenase yields NADH and oxaloacetate. This reaction is a rare oxidation reaction that is energetically unfavorable. Conversion of malate to oxaloacetate is the only energy "bump" to be gotten over in the citric acid cycle and that is readily accomplished thanks to the 'pulling' of the citrate synthase reaction, which keeps oxaloacetate concentrations low.

18. The citric acid cycle can be regulated allosterically in several places, but the most important regulation of the cycle is probably the amount of NAD^+ and FAD that is available. NAD^+ (and FAD) is essential for the cycle to operate and it is essential for the pyruvate dehydrogenase complex reaction to occur. This relates to metabolic control, as

will be more obvious in the sections on electron transport and oxidative phosphorylation.

19. When all of the NADHs and $FADH_2$s of the citric acid cycle are converted to ATP, the cycle yields 30- 38 ATPs per molecule of glucose (depending on how you count them), compared to 2 for glycolysis (under anaerobic conditions). The citric acid cycle is thus an incredibly efficient producer of energy for the cell.

20. Many factors combine to regulate metabolism through the citric acid cycle. All of these ultimately come down to energy needs. Most are manifested through the availability or lack of NAD^+. When NAD^+ is lacking (high NADH levels), the cycle will be inhibited. When NAD^+ levels are high (low NADH levels), the cycle is favored. Oxygen is a limiting reagent needed to keep the citric acid cycle turning. This is because oxygen is required ultimately for the conversion of NADH back to NAD^+.

Remember that NAD^+ is required for three reactions of the citric acid cycle. If any one of these reactions is stopped, the cycle grinds to a halt. While glycolysis can use fermentation to get around conditions lacking oxygen, the citric acid cycle cannot.

21. The pyruvate dehydrogenase complex is regulated by substrate level regulation (NAD^+/ NADH) and allosteric regulation (ATP inactivates, AMP and ADP activate). The complex is also regulated by covalent modification - phosphorylation/ dephosphorylation. The relevant enzymes are a kinase, which phosphorylates pyruvate dehydrogenase (inactivating it) and a phosphatase, which dephosphorylates pyruvate dehydrogenase, activating it.

22. Pyruvate dehydrogenase is sensitive to arsenite and mercury, due to the fact that these compounds will react with the sulfur

The puzzle was clear on the shore
When the crew team's most talent rower
Got caught in a bind
To make up his mind
When he just couldn't pick either oar

atoms in lipoamide of E_3 of the pyruvate dehydrogenase complex. Treatment of lipoamide with BAL extracts arsenite from the lipoamide, due to the fact that BAL also contains sulfurs to which the arsenite binds.

23. Anaplerotic reactions are involved in "filling up" the intermediates of metabolism, which are needed for multiple purposes. For example, oxaloacetate may be used up in making aspartic acid and must be replenished. One mechanism for this may be conversion of glutamic acid into alpha ketoglutarate, with subsequent conversion to oxaloacetate. Citric acid cycle intermediates are involved in metabolism of amino acids, fatty acids, nucleotides, and sugars.

24. Oxaloacetate is important in many metabolic pathways. It can be converted to glucose in gluconeogenesis, to aspartate by transamination, and to citrate in the citric acid cycle. Both oxaloacetate and alpha ketoglutarate are important anaplerotic intermediates.

Biologists will not stand by
And bid all the gophers goodbye
We're all interlinked
So if they go extinct
The hole ecosystem might die

25. The glyoxylate cycle is a pathway related to the citric acid cycle that occurs in plants and bacteria. It requires two enzymes not found in animals (in addition to the normal enzymes of the citric acid cycle). The enzymes unique to the glyoxylate cycle are isocitrate lyase (catalyzes cleavage of isocitrate to glyoxylate and succinate) and malate synthase (catalyzes linkage of acetyl-CoA to glyoxylate to form malate).

In the office it was not a thrill
When my dentist had three teeth to fill
Things couldn’t get worse
When he said to the nurse
“All right, I think you know the drill”

26. Because of decarboxylation in the citric acid cycle, no net synthesis of glucose can occur via the citric acid cycle. On the other hand, plants and bacteria that have the enzymes of the glyoxylate cycle are able to form glucose in net amounts from acetyl-CoA because they can bypass the decarboxylation reactions of the citric acid cycle and convert acetyl-CoA into useful material.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Lipids and Membranes

1. Membranes are composed of a lipid bilayer and other molecules, such as cholesterol. The lipid bilayer contains two main categories of molecules - glycerophospholipids and sphingolipids.

2. Glycerophosphlipids contain glycerol, phosphate and one or more fatty acids. The fatty acids in the molecules of the lipid bilayer are either saturated (no double bonds) or unsaturated (contain one or more double bonds). Double bonds in biological fatty acids are almost exclusively in the *cis* configuration, resulting in a bent shape for unsaturated fatty acids.

3. Glycerophospholipids (also called phosphoglycerides) are related to fats in having a glycerol backbone and two fatty acids, but they differ from fat in having a phosphate located in position #3 of the glycerol. Naming of glycerophospholipids is generally as "phosphatidyl-X" where X is the name of the molecule attached to the phosphate. Examples include phosphatidylserine, etc.

4. If there is no other molecule attached to the phosphate on the glycerophospholipids described above in #3, you have phosphatidic acid. Phosphatidic acid is an important intermediate in synthesis of phosphatidyl lipids, as well as fats.

At the bookstore I asked timidly
For some help to get phobia-free
The clerk pointed to
A brand new review
But I fear that it may not help me

5. Sphingolipids are molecules related to glycerophospholipids that are based on sphingosine. Sphingomyelin is a component of the myelin sheath of nerve cells. Sphingolipids containing a

single sugar are called cerebrosides and sphingolipids containing a complex carbohydrate moiety are called gangliosides. Sphingolipids are prominent components of the membranes of nerves and brain tissue.

6. Steroids are lipids that are not derived from fatty acids. In animals, steroids are derived from cholesterol. Cholesterol is found in the membrane of cells and is important for membrane stability. Cholesterol is prominent in brain membranes - up to 14% of the dry weight of brain.

7. A lipid bilayer has a polar exterior facing water and a non-polar interior. As such, the membrane provides a good barrier to both polar and non-polar substances. In contrast to the lipid bilayer, fatty acids aggregate into a micelle.

8. In addition to glycerophospholipids and sphingolipids, membranes contain proteins, glycoproteins, and glyolipids. Four types of membrane proteins are integral (protein projects through both sides of the membrane), peripheral (protein projects into only one side of the membrane), anchored (protein is linked to a molecule embedded in the lipid bilayer, or associated (protein associates by hydrogen bonding with an integral membrane protein.

9. Integral membrane proteins are difficult to remove from membranes, but peripheral and associated membrane proteins are not.

10. Membranes provide a barrier between the cell and the external environment. Membranes provide a barrier to passage of many molecules, including molecules, such as sugar that the cell could use for food.

11. Integral membrane proteins span into and/or across the plasma membrane and thus must have both hydrophilic and hydrophobic portions that interact appropriately with the same portions of the plasma membrane. By contrast, peripheral membrane proteins are found in association with membranes, such as by interacting with an integral membrane protein. Anchored membrane proteins are proteins attached to a molecule (like a fatty acid). The molecule is embedded in the lipid bilayer and thus the protein is anchored to it.

12. Bacteriorhodopsin is an integral membrane protein that uses light, chemistry, and mechanics to move protons across a membrane barrier.

13. One can assemble artificial lipid bilayers containing compounds as a means of delivering materials into cells. These artificial systems are called liposomes. Liposomes can carry substances and when the membrane of the liposome fuses with a cell membrane, the contents of the liposome are delivered into the cell. This is a useful way of getting compounds into cells that are not easily transported across the cell membrane in other ways.

14. Membrane-spanning proteins have alternating regions of non-polar membrane crossing regions interrupted by polar short sections joining the non-polar regions. Consequently, one can use a computer to examine the amino acid sequence of a protein and predict reasonably accurately if the protein is a membrane protein or not.

15. Cellular membranes are somewhat fluid in nature. The fluidity of membranes is related to their composition - shorter, more unsaturated fatty acids make for membranes that retain fluidity at lower temperatures

compared to longer, saturated fatty acids. Fish membranes, for example are full of unsaturated and polyunsaturated fatty acids, which makes sense because fish membranes must be fluid at fairly low temperatures.

16. Biological membranes can be in a fluid or a more solid state. The midpoint of the conversion between the solid and the fluid state is referred to as the T_m. Cholesterol is often found in membranes. Though it does not change the T_m of a membrane cholesterol does widen the range of the transition temperature between solid and fluid state.

17. Unsaturated fatty acids in glycerophospholipids and sphingolipids tend to lower the T_m of a membrane. Saturated fatty acids tend to raise the T_m. Shorter fatty acids also lower T_m, but longer ones raise it.

18. The Fluid Mosaic Model explains the fluidity of cellular membranes.

19. Not all molecules move into a cell through specific protein receptors. An example of such an exception is cholesterol, which enters cells via LDLs that attach to a receptor on the cell's surface. The entire LDL with the cholesterol is taken into the cell in a process called endocytosis.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Membrane Transport

1. Diffusion is a process in solutions where molecules move from a high concentration to a low concentration. Active transport occurs when a least one molecule is moved across a membrane from a low to a higher concentration. This takes energy.

2. We break transport across membranes into two main categories - 1) passive transport (diffusion driven, so materials move only from high concentration to lower concentration and don't require outside energy), and 2) active transport (an energy-requiring process that moves at least one molecule from a low concentration to a higher concentration - this is counter to simple diffusion).

The baker of bread has succeeded
His quotas today all exceeded
The reason he's blessed
You probably guessed
He had everything that he kneaded

3. Active transport moves at least one molecule in the opposite direction of where diffusion would operate (that is, active transport moves at least one molecule from a low concentration to a higher concentration).

4. ATP is a primary energy source for active transport, but there are other sources, as well (see below). The term 'pump' is used to describe the protein component of an active transport system. Pumps that move two molecules in the same direction across a membrane are called symports (or synports), whereas pumps that move two molecules in opposite directions across a membrane are called antiports. Pumps are called electroneutral if their action does not result in a net change in charge and electrogenic if their action changes the charge across the membrane as a result of their action.

The heir didn't think it was great
With his uncle now at heaven's gate
He got zero stocks
Just a hundred old clocks
So he's now winding up his estate

5. An example of a passive transport system is a glucose transporter in blood cells that simply lets glucose diffuse into cells. No energy other than that provided by the process of diffusion is required for that particular transporter. Other glucose transporters in other cells are active in that they use energy to move glucose against a concentration gradient.

6. An example of a passive transport system is a glucose transporter in blood cells that simply lets glucose diffuse into cells. No energy is required for that particular transporter. Other glucose transporters in other cells are active in that they use energy to move glucose against a concentration gradient.

7. P-type ATP-using transport systems use phosphoaspartate as a covalent intermediate in their mechanism of action.

8. The mechanism of transport of the Ca/ATPase pump includes binding of ATP and the relevant ions (calcium, in this case), transfer of phosphate from the ATP to the protein (making phosphorylaspartate), conformational change in the protein

causing movement of the ions across the membrane, hydrolysis of the phosphate from an aspartic acid side chain in the protein, a second conformational change to bring the protein back to its original state.

9. Another P type ATPase is the Na$^+$/K$^+$ ATPase. The Na$^+$/K$^+$ ATPase transports three sodiums out of the cell and two potassiums in during each cycle. This is an electrogenic transport mechanism and uses hydrolysis of ATP to drive the process. Movement of Na$^+$ and K$^+$ is essential for the cell being able to maintain osmotic balance. The Na$^+$/K$^+$ ATPase is called an antiport because it moves molecules in opposite directions.

10. Another class of transporter proteins that use ATP to move molecules is the ABC transporters. An example is the Multidrug Resistance Protein that is involved in the resistance of cancer cells to chemotherapy agents. They act by binding the compound first. This causes a conformational change in the protein that allows ATP to bind. Binding of ATP causes the protein to 'evert' (move its opening from one side of the membrane to the other). This has the effect of moving the bound compound to the outside of the cell. After this happens, ATP is hydrolyzed to change the protein to evert again and change back to its original conformation (opening facing inwards).

11. The Na$^+$/Ca^{++} exchange pump is a secondary transporter. It uses movement of Na$^+$ in to cells to be a driving force for pumping Ca^{++} out. Remember than Ca^{++} stimulates muscular contraction. If Ca^{++} is not pumped OUT, its concentration in muscle cells remains high, stimulating contraction. Digitoxigenin is a poisonous compound from foxglove that binds the Na$^+$/K$^+$ ATPase, preventing development of a Na$^+$ gradient. As a consequence, digitoxigenin increases Ca^{++}

concentration, since Ca^{++} pumping requires a Na^+ gradient. Digitoxigenin is used as a heart stimulant, but is a dangerous poison that must be properly used.

12. Proteins that move more than one chemical in the same direction across a membrane are called symports (synports). Those that move them in opposite directions are called antiports. If a net charge difference arises as a result of the movement, the system is referred to as electrogenic. If no charge difference arises, they are called electroneutral.

13. Nerve cells use the gradient of Na^+ and K^+ built up by the Na^+/K^+ ATPase pump to transmit signals. In nerve transmission, special "gates" open and close to allow Na^+ to diffuse into nerve cells and K^+ to diffuse out of nerve cells.

14. The first step in nerve transmission involves opening of Na^+ gates. These allow Na^+ to diffuse into the cell, since Na^+ concentration is higher outside of cells than inside. Movement of the positively charged sodium ion causes a change in the electrical potential of the cell near the Na^+ gate. To compensate for the voltage change, the K^+ gates open and Na^+ gates close, allowing K^+ to flow out of the cell. This results in an overcompensation of the voltage. The K^+ gates close and the region where the original movement of ions occurred recovers. During this time, no nerve signal can be transmitted at that point.

15. The nerve signal is transmitted as a consequence of the initial movement of Na^+ into the cell. Before it can be pumped out, some of the sodium diffuses down to the next Na^+ gate and the change in the voltage environment causes it to open and trigger the same events as

occurred in the last step. Thus, the signal moves from one junction to another to another, ultimately arriving at the end of the axon.

16. Tetrodotoxin is a neurotoxin because it inhibits the action of nerve cells. It is found in the puffer fish and it blocks the Na^+ gates.

17. Channels (gates) are made by protein molecules in the membranes of cells. Channels are generally very specific for what they will allow to pass through them. Glucose channels, for example are fairly specific for glucose. Sodium and potassium channels are very specific for each respective ion.

18. Ion specificity is accomplished by two mechanisms. The first is physical. If an ion is too big to fit in a channel, it is excluded. This is the case of the sodium channel, which excludes potassium ions because they are too big.

19. The second mechanism of specificity is energy. An example is the potassium channel, which excludes sodium ions. In this case, the channel allows larger ions (potassium) to pass through, but blocks smaller ions, like sodium ions.

20. The mechanism of exclusion of the potassium channel relates to the energies of solvation of each ion. For potassium ions, the energy of desolvation of the ion as it enters the channel is overcome by the energy of resolvation as it enters the channel. Thus, entry of potassium ions is energetically favored. This is due to the geometry of the potassium channel closely matching the dimensions of the potassium ion.

21. When sodium ions try to enter the channel, their energy of desolvation is greater than is realized by their resolvation in the

channel. Thus, they do not enter. Their energy of resolvation not as favorable due to their ion sizes not matching the dimensions of the potassium channel.

22. After the nerve has "fired" the gradient must be restored and this is the job again of the Na^+/K^+ ATPase.

23. After a nerve "signal" has moved along the entire length of a nerve cell, it must move to the adjacent cell. This requires a neurotransmitter. Neurotransmitters are small molecules encapsulated in vesicles (synaptic vesicles) that are released from the nerve cell carrying the signal (presynaptic membrane) to the adjacent nerve cell (postsynaptic membrane). Acetylcholine, for example, is released in presynaptic vesicles and when the contents (acetylcholine) bind receptors on the postsynaptic membrane, channels open to allow sodium and potassium ions to move as before, causing the next nerve cell to start an action potential.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Mitochondria and Energy

1. Mitochondria are the locations in cells where oxidation/reduction and ATP synthesis occurs, along with metabolism. Mitochondria have distinctive structural features, including an inner membrane that is impermeable to protons, infoldings of the inner membrane called cristae, an outer membrane that is not very impermeable, an intermembrane space between the inner and outer membrane, and the matrix. This last "structure" is simply the fluid in the inner mitochondrion and it is here where the enzymes of the citric acid cycle and fatty acid oxidation are found.

2. Remember that for every oxidation, there is an equal and and opposite reduction. Loss of electrons by one molecule means gain of them by another one. Oxidation is a process that involves the loss of electrons. Reduction is a process that involves the gain of electrons.

3. Electrons are carried to the electron transport system in the mitochondria by NADH and $FADH_2$.

At the magic show people gave stares
To the man doing tricks with a flare
Some were appalled
Cuz they thought he’d go bald
From constantly pulling out hares

4. Mitochondria are the site of electron transport and oxidative phosphorylation.

5. Electrons from NADH enter the electron transport system through complex I.

6. Electrons from $FADH_2$ enter the electron transport system through complex II.

The man in the dark walked unsighted
Dead batteries seemingly blighted
He just hit a wall
He could not see at all
His flashlight was simply delighted

7. Coenzyme Q (CoQ) accepts a pair of electrons from either complex I or complex II and passes electrons singly to cytochrome c through complex III. Thus, coenzyme Q acts as a "traffic cop" for electrons.

8. The sequence of electrons passing from coenzyme Q is as follows: Coenzyme Q -> Complex III -> Cytochrome c -> Complex IV -> oxygen (to form water)

9. Oxygen is thus the terminal electron acceptor and is a limiting compound during periods of heavy exercise.

The award ceremony revealed
When the big envelope was unsealed
The scarecrow got buzz
The reason because
He’s just out standing in his field

10. If oxygen is not available, electrons will NOT pass through the electron transport system and NADH and $FADH_2$ will not be reoxidized. For these reasons, the citric acid cycle will not run either. This is part of metabolic control.

11. Several compounds inhibit electron transport - rotenone (an insecticide) and amytal block all action of Complex I. Antimycin A blocks all action of Complex III. Cyanide, azide, and carbon monoxide block all action of complex IV.

12. Movement of electrons through Complex III is known as the Q cycle. This cycle begins with the binding of two molecules of CoQ (QH_2 and Q) to Complex III. QH_2 has two electrons and two protons. Q has neither.

13. After QH_2 and Q bind, QH_2 sends one electron to Q, creating Q^- and one electron to cytochrome C. The two protons QH_2 was carrying are expelled into the intermembrane space. This converts QH_2 to Q. Both cytochrome C and Q leave the complex, but Q^- remains behind.

14. Next, another QH_2 and another cytochrome C binds to Complex III. QH_2 sends one electron to Q^-, creating Q^{-2} and one electron to cytochrome C. It also expels its two protons to the intermembrane space and becomes Q. Then Q^{-2} extracts two protons from the matrix and becomes QH_2. Last, cytochrome C, QH_2, and Q all leave the complex.

15. Electron transfer through complex IV occurs one electron at a time (since one electron arrives at a time from cytochrome c). Interruption of electron flow can result in production of reactive oxygen species. Cellular enzymes, such as superoxide dismutase and catalase (see below) help to deactivate reactive oxygens.

The overweight man couldn’t bear
Each diet led him to despair
So he raced to the door
Of the nearest paint store
When he heard you could get thinner there

16. In electron flow through complex IV, the first electron is

transferred to copper and the second one is transferred to iron. Oxygen then binds to the iron first, followed by formation of a peroxide bridge between the iron and copper atoms. Addition of a third electron (to the oxygen on the copper) and binding of a proton from the matrix causes the O-O bond to be cleaved. A fourth electron then reduces the oxygen on the iron and a proton binds from the matrix as well. Last, two protons from the matrix bind to the hydroxyls on the iron and copper, forming two water molecules, which are released and the cycle is complete.

17. During electron movement through Complex IV, four protons are taken from the matrix and combined with oxygen to form two water molecules. In addition, four other protons are taken from the matrix by the complex and pumped outside the mitochondrial matrix. As a consequence, the proton numbers in the matrix decrease by 8 during the process. The proton numbers outside the mitochondrion INCREASE by four in the process, so the net difference is 12 protons just for movement through complex IV.

18. ATP is created in oxidative phosphorylation by the movement of protons back into the mitochondrial matrix through complex V (also called the ATP synthase).

19. Two essential functions of electron transport - 1. Pump protons out of mitochondrial matrix and 2. Reoxidize NADH and $FADH_2$ to NAD and FAD, respectively. In healthy, normal cells, oxidative phosphorylation is tightly coupled to electron transport. Stopping electron transport will ultimately stop oxidative phosphorylation in tightly coupled mitochondria.

There’s one indisputable fact
The train’s engineer should react
Ignoring transponders
As his mind slowly wanders
Is just how he got so side tracked

20. The chemiosmotic hypothesis, originally proposed by Peter Mitchell, explains how mitochondria make ATP in oxidative phosphorylation. Important aspects of it include:

a. Intact inner mitochondrial membrane
b. Electron transport creates a proton gradient
c. ATP is made by movement of protons back into the mitochondria

21. Tight coupling of electron transport and oxidative phosphorylation requires at a practical level that the mitochondrial inner membrane remain impermeable to protons, except for those that enter via the ATP synthase and result in ATP production.

22. The ATP synthase consists of a turbine-like structure containing 3 sites called Loose (L), Tight (T), and Open (O).

Functions of these forms include

L - Holds ADP and P_i in preparation for ATP formation
T - Causes ADP and P_i to join and form ATP
O - Releases ATP formed in T and binds ADP + P_i

23. Movement of protons through the ATP synthase cases rotation/conformational changes in the complex that result in formation of ATP from ADP and P_i. Conversions in the process occur as follows:

O goes to L
L goes to T
T goes to O

24. Mitochondria which are "tightly coupled" have intact membranes AND the only way protons get back into the matrix is by passing through Complex V. If you poke a hole in the membrane (using DNP or an uncoupling protein, such as found in brown fat), protons can leak back in without making ATP. This has the effect of generating heat AND burning up energy sources (like glucose and fat). DNP is a dangerous compound that killed people who tried to use it to lose weight.

Near a cow everyone should beware
Of the gas they release everywhere
It’s the reason they say
On almost any day
That a farm smells just like dairy air

25. When mitochondria are tightly coupled, metabolic (respiratory) control exists. This means that electron transport will stop if oxidative phosphorylation stops, since the protons don't come back in, so the proton gradient gets very high, stopping the pumping of protons. When electron transport stops, NADH accumulates and the citric acid stops. Conversely, if one stops electron transport with cyanide, oxidative phosphorylation will stop

In political science Ms Parks
Spoke her mind and created some sparks
She said that her grade
Was surely waylaid
Because she had such lousy Marx

shortly because the proton gradient is lost when no protons are pumped.

26. When mitochondria are uncoupled (by poking a hole in them to let protons leak back into the matrix without passing through Complex V), electron transport is no longer limited by oxidative phosphorylation and runs amok. That is why heat is generated. Protons are pumped, but they fall back in through the hole in the mitochondrial inner membrane. No ATP is made. NADH is rapidly converted to NAD^+, so the citric acid cycle and other pathways run rapidly.

27. The compound 2,4 DNP (dinitrophenol) was marketed as a miracle diet drug about a century ago. It kills because it destroys the proton gradient of mitochondria without allowing synthesis of ATP.

28. Things that affect respiration are ADP (necessary for the Complex V to function), oxygen (necessary for electron transport to function), NADH (source of electrons for electron transport), and NAD^+ (needed for citric acid cycle).

29. NADH cannot cross the inner membrane of the mitochondrion, as there is no protein to move it. Electrons make it into the mitochondria by means of shuttles. Insect muscles use a glycerol-3phosphate/DHAP shuttle that transfers electrons from NADH ultimately to $FADH_2$. Mammalian systems, by contrast, employ a malate/aspartate system that converts oxaloacetate to malate (carrier of electrons) which gets transported by a transport protein.

In heaven an organized plan
Uses numbers wherever they can
Mr. Thirteen said “I’m
Forever a prime.”
A most indivisible man

Once inside the matrix, malate transfers electrons to NAD^+, creating NADH and oxaloacetate.

30. Superoxides are reactive oxygen species generated as a byproduct of electron transport (among other things). They react with whatever they encounter, so can be damaging to DNA, proteins, etc. Superoxide dismutase is an enzyme employing a ping-pong mechanism that works in two steps to destroy two superoxide molecules, while producing hydrogen peroxide.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Photosynthesis

1. Photosynthesis is a process in plants and some bacteria that use energy from the light of the sun to synthesize glucose using carbon dioxide and water as starting reagents. It accomplishes this in a multistep process that is divided into two phases, called the light reactions (require light) and the dark reactions (don't require light).

2. Photosynthesis occurs in plants in organelles called chloroplasts. The thylakoid disks of the chloroplast are the sites where the light reactions of photosynthesis and the stroma are the locations of the dark reactions.

Perhaps it was all the demands
Disrupting the cannibal's plans
Or maybe the stress
That he tried to suppress
That made him go throw up his hands

3. Molecules involved in the capture of light energy are known as the chlorophylls. These molecules contain a porphyrin ring (like hemoglobin) with a magnesium ion at the center (instead of the iron molecule found in hemoglobin).

4. In the light reactions of photosynthesis, 1) electrons are removed from water (producing oxygen); 2) ATP is produced by the process of photophosphorylation as electrons pass through the membrane of the thylakoids; and 3) NADPH is produced from NADP$^+$ in the final reduction reaction. NADP$^+$ is therefore the terminal electron acceptor in photosynthesis, whereas water is the electron source for the process.

5. Movement of electrons through the electron transport chain in the thylakoid membrane causes protons to be pumped INTO the thylakoid. This creates a proton gradient (higher proton concentration in the thylakoid than the stroma). Protons in the thylakoid move outside through a proton translocating ATP synthase (PTAS) complex (same general structure as the mushroom-like complex with the same function in mitochondrial membranes). As protons move through the PTAS, ATP is generated from ADP (photophosphorylation).

It is true that Cher got a small shiner
When she hurried to see her designer
Of her speed police said
It wasn't widespread
Thus was the star's inertia minor

6. There are two photosystems (I and II) that act together to produce the reactions of photosynthesis. Photosystem II is involved in the first set of reactions. Here electrons from the first porphyrin ring complex are excited by light. As these electrons are passed to the electron transport chain of the membrane, the ring extracts electrons from water, creating oxygen.

7. Movement of electrons through the electron transport chain in the thylakoid membrane causes protons to be pumped INTO the thylakoid. This creates a proton gradient (higher proton concentration in the thylakoid than the stroma). Protons in the thylakoid move outside through a proton translocating ATP synthase (PTAS) complex (same general structure as the mushroom-like complex with the same function in mitochondrial membranes). As protons move through the PTAS, ATP is generated from ADP (photophosphorylation).

8. Electrons released from photosystem II eventually reach photosystem I where they are excited by sunlight of a different wavelength. These newly excited electrons are passed to ferredoxin and ultimately to $NADP^+$, creating NADPH, the final product of the light reactions.

9. Quinones are molecules that help carry electrons in the thylakoid membrane (and mitochondrial membrane too).

10. The dark reactions of photosynthesis are where the glucose is synthesized. Thus, we can think of the light reactions as the ones where the energy necessary for making glucose is stored

Otter Nonsense

In Russia the pundits expound
Many theories whene'er they're around
They'd recover much faster
From World War disaster
If they hadn't kept Stalin around

up (charging a battery) and the dark reactions as the ones that use energy from the light reactions to store that energy in another form - glucose.

11. The dark reactions of photosynthesis are also known as the Calvin cycle in honor of their discoverer, Melvin Calvin.

12. In the dark reactions, carbon dioxide is removed from the atmosphere in a process called fixation. Carbon dioxide is first covalently attached to ribulose1,5bisphosphate (Ru1,5BP) to form a six carbon intermediate that immediately breaks down to form two molecules of 3-phosphoglycerate (3-PG) for each molecule of carbon dioxide combined with Ru1,5BP. This reaction is catalyzed by the enzyme with the acronym RuBisco.

13. 3-PG is an intermediate in glycolysis and gluconeogenesis. Phosphorylation and reduction of it leads to glyceraldehyde-3-phosphate (G3P).

14. The pathway described to this point is that taken by plants known as C_3 plants, by virtue of the fact that the first molecule made after fixation of carbon dioxide has three carbons. Another group of plants, known as C_4 plants, fixes carbon dioxide to PEP and forms (surprise!) a four carbon molecule, oxaloacetate. Ultimately, this four carbon molecule is decarboxylated in another part of the plant, releasing carbon dioxide once again. This newly released carbon dioxide enters the Calvin cycle (same reactions here as C_3 plants). This unique delivery system allows C_4 plants to efficiently deliver CO_2 to where it is used and allows them to avoid water loss. It may also allow them to be more efficient in that the rubisco reaction is occurring in the plant at a location where the oxygen concentration is lower than it is where the rubisco reaction occurs in C_3 plants.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Lipid and Steroid Metabolism

1. Phosphatidic acid is an immediate precursor of CDP-diacylglycerol, which is a precursor of the various glycerophospholipids. CTP combines with phosphatidic acid to yield a pyrophosphate and CDP-Diacylglycerol. Activation by CDP yields a high energy activated intermediate that can be readily converted to phosphatidyl glycerophospholipids.

2. From CDP-diacylglycerol, phosphatidyl serine can be made, as can phosphatidyl ethanolamine and phosphatidyl choline. Synthesis of phosphatidyl choline from phosphatidyl ethanolamine requires methyl groups donated by S-Adenoysyl-Methionine (SAM). Loss of the methyl groups by SAM yields S-Adenosyl-Homocysteine.

3. Phosphatidyl ethanolamine (and phosphatidyl choline - derived from phosphatidyl ethanolamine) can both be made independently of phosphatidic acid biosynthesis. For this pathway, CDP-ethanolamine is the activated intermediate and the phosphoethanolamine of it is added to diacylglycerol to form phosphatidylethanolamine. Phosphatidyl choline can be made by the same methylation scheme in point 4.

4.Sphingolipids are synthesized beginning with palmitoyl-CoA and serine. Addition of a fatty acid to the amine group yields a ceramide. Addition of sugars to a ceramide yields either a cerebroside (single sugar added) or a ganglioside (complex sugar added).

5.Deficiencies in enzymes that degrade sphingolipids (particularly cerebrosides and gangliosides) are linked to neural disorders. One such disorder is Tay-Sachs disease.

6. Cholesterol is an important component of membranes, particularly in the brain. Cholesterol can be synthesized totally from acetyl-CoA.

7. Steroids include all compounds synthesized from cholesterol. This includes steroid hormones, vitamin D, bile acids, and other related compounds.

The haberdasher's remark was misread
When he looked towards his friends and then said
"You guys should all know
That to be status quo
I have to go right on a head"

8. Isoprenes (also called isoprenoids) include the steroids and other compounds, such as Vitamin A and E that are also made from isoprene units.

9. Isoprene - 5 carbon units - 1) isopentenyl pyrophosphate and dimethylallyl pyrophosphate. Each is made from acetyl-CoA molecules. Synthesis of isopentenyl pyrophosphate and dimethylallyl pyrophosphate comes from mevalonate, which, in turn, comes from HMG-CoA, so isoprenoid biosynthesis overlaps partly with ketone body synthesis.

The writing instructor would show
All her students the writing rules though
She seemed quite the fool
When she read them the rule
"Double negatives are a no-no"

10. The most important enzyme in cholesterol biosynthesis is HMG-CoA reductase. It converts HMG-CoA to mevalonate and is the primary regulatory enzyme in cholesterol biosynthesis. Cholesterol is a feedback inhibitor of the enzyme.

11. Reaction of isopentenyl pyrophosphate with dimethylallyl pyrophosphate yields a 10-carbon intermediate, geranyl pyrophosphate.

12. Reaction of geranyl pyrophosphate with isopentenyl pyrophosphate yields a 15 carbon intermediate - farnesyl pyrophosphate.

13. Reaction of two farnesyl pyrophosphates yields a 30 carbon intermediate - squalene.

14. Further reaction of squalene yields a cyclic intermediate, lanosterol. Synthesis of cholesterol from lanosterol requires an additional 19 steps. From cholesterol, one can synthesize the bile salts, which are useful in solubilizing fat in the diet. Cholesterol also leads to synthesis of the steroid hormones.

15. Cholesterol in the body is either there as a result of 1) synthesis; 2) diet; or 3) storage/recycling.

16. In the digestive system, bile acids and mechanical agitation of the stomach help emulsify fats.

17. In the intestines, hydrolysis of fatty acids from fats by lipases (pancreatic lipase, for example), yield soapy like substances that help emulsify lipids for transport across the intestinal wall.

18. After movement across the intestine, lipids are packaged in chylomicrons. They move through the lymph system into the capillaries where they get stuck.

19. Action of lipoprotein lipase in the capillaries removes some of the fat from the chylomicrons and they shrink in size and exit the capillaries and move to the liver. They are taken up there and released, when needed as VLDLs. These travel the blood system and get converted to IDLs and ultimately LDLs. Note that cholesterol ONLY gets into cells via receptor-mediated endocytosis of the LDL at the target cells.

20. The liver is involved in sensing the body's need for lipids via LDL receptors. If it senses lipids are needed, VLDLs are packaged by the liver and released into the bloodstream. These get degraded by lipases and other enzymes to IDLs and LDLs. If the liver LDL receptor cannot detect LDLs in the blood, it continues to release more lipids in lipoprotein complexes, thus elevating the LDL concentration.

It is true that the student's decision
Getting glasses helped him to envision
Mathematical ties
And soon realize
They helped him two ways with division

21. Defects in liver LDL receptors, such as found in familial hypercholesterolemia (a genetic disease) are one cause of high blood cholesterol. Other factors to consider include dietary cholesterol, cholesterol synthesis rate, and efficiency of recycling cholesterol. Statins are drugs that inhibit HMG-CoA reductase and reduce cholesterol by inhibiting its synthesis.

22. LDLs are called bad cholesterol because in high amounts, they can lead to formation of atherosclerotic plaques. These arise due to oxidative damage of unsaturated fatty acids in the LDL by reactive oxygen species. The immune system attacks these damaged LDLs and the resulting complex can lead to the formation of foam cells (rich in cholesterol) and finally a physical block to the flow of blood (plaque).

23. Factors increasing LDLs include smoking, obesity, and saturated fats in the diet.

24. Opposing the LDLs are the HDLs (good cholesterol). They are associated with removing cholesterol from the bloodstream and giving feedback to the liver to reduce the output of VLDLs. High levels of HDLs correlate with reduced incidences of atherosclerosis. Factors increasing HDLs include exercise and factors decreasing them include obesity and smoking.

25. If a person has high cholesterol, the first approach is to see if levels can be brought down by dietary changes. If they cannot, then drugs are used to stop 1) endogenous synthesis and/or 2) recycling.

26. SREBP (Steroid response element binding protein) is a protein that plays a role in controlling whether or not HMG-CoA reductase is made. When cholesterol is abundant, SREBP is found in the membrane of the endoplasmic reticulum linked to another protein called SCAP through the regulatory (REG) region of SREBP. When cholesterol is abundant, SCAP-SREBP-A is held in the endoplasmic reticulum's membrane by a protein called INSIG

27. When cholesterol levels in the cell fall, the SCAP/SREBA complex loses its connection to INSIG and moves to the Golgi Complex where a serine protease cleaves SREBP-A to release SREBP-B. Next, SREBP-B migrates in the membrane to a metalloprotease, which clips the DNA binding region to free SREBP-C (a transcription factor) from the membrane-bound portion. The SREBP-C has a DNA binding region then travels through the cytoplasm and enters the nucleus, where it binds to the promoter region (called SRE) in front of the HMG-CoA Reductase. This causes HMG-CoA

Reductase to be synthesized and cholesterol synthesis can then begin.

28. HMG-CoA reductase can also be broken down when cholesterol is abundant. This occurs through action of another INSIG. This INSIG carries an enzyme to ubiquitinylate (put ubiquitin onto) the HMG-CoA reductase. Ubiquitin is a "flag" to the cell to digest the protein it is attached to with a protease, thus destroying it.

If a chicken should ere get the notion
Crossing roads by its own locomotion
There’s no need to ask why
That the birdie passed by
It is simply some poultry in motion

29. Phosphorylation of HMG-CoA reductase is also regulated by feedback inhibition (allosteric) and phosphorylation (covalent modification). Thus, HMG-CoA reductase can be regulated in multiple ways - synthesis, degradation, phosphorylation, allosterism.

30. Cholesterol is a precursor of the bile acids that are important in the digestive system for solubilizing dietary fat. Two examples include glycocholate and taurocholate.

31. Cholesterol is also a precursor of the steroid hormones. There are five groups of steroid hormones. These are all derived from pregnenolone and include androgens (male sex hormones), estrogens (female sex hormones,which are derived from male sex hormones), mineralocorticoids (regulation Na/K and blood pressure), glucocorticoids (fat/protein degradation & swelling/ inflammation), and progestagens (maintenance of pregnancy) .

32. Conversion of androgens to estrogens requires action of an enzyme known as aromatase. Since some tumors are stimulated by estrogens, such as estradiol, inhibition of them by aromatase inhibitors is a strategy of some chemotherapies.

33. Vitamin D is derived from cholesterol (ultimately). A reaction converting 7-dehydrocholesterol to Previtamin D_3 requires ultraviolet light. Conversion of Previtamin D_3 to Vitamin D_3 occurs spontaneously. Conversion of Vitamin D_3 to calcitrol requires an enzyme and the enzyme is regulated to control how much calcitrol is made. Calcitrol is the active form of vitamin D. Vitamin D is involved in regulating uptake of calcium and phosphorus, which are important for healthy bones. It may also play roles in mental health and has been shown to have anti-cancer properties.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Fatty Acid Metabolism

1. In the digestive system, bile acids and mechanical agitation of the stomach help emulsify fats.

2. In the intestines, hydrolysis of fatty acids from fats by lipases (pancreatic lipase), yield soapy like substances that help emulsify lipids for transport across the intestinal wall. On the other side of the wall, they are reassembled back into fats and these are packaged in the chylomicrons.

3. Breakdown of fats occurs by action of enzymes known as lipases and these may be extracellular or intracellular.

4. Cells that store fat are known as adipocytes.

5. Fatty acid oxidation and fatty acid synthesis are almost identically the chemical reverse of each other.

6. Fats are broken down to fatty acids and glycerol by enzymes known as lipases. One of these, hormone sensitive triacylglycerol lipase, is the only regulated enzyme of fat or fatty acid breakdown. It is located in fat-storing cells called adipocytes.

7.Triacylglycerol lipase action cleaves the first fatty acid off of a fat and this step is necessary before the other lipase can act to remove the other fatty acids from a fat.

8. Glycerol, is the only part of a fat that can be made into glucose (via gluconeogenesis). Fatty acids travel in the bloodstream carried by serum albumin.

9.Fatty acid oxidation occurs in the matrix of the mitochondrion. In the cell, fatty acids are attached to CoA and then at the mitochondrion, the CoA is replaced by carnitine. Inside the mitochondrial matrix, the carnitine is replaced by CoA again.

10. Fatty acid oxidation proceeds in steps that mirror steps in the citric acid cycle. These include dehydrogenation, hydration, and oxidation. In the last step, a thiolytic cleavage releases acetyl-CoA and a fatty acid two carbons shorter.

11. The dehydrogenation and oxidation reactions yield reduced electron carriers ($FADH_2$ and NADH). The double bond formed in the first dehydrogenation reaction is in the *trans* form. The hydration yields a hydroxyl group on the third carbon from CoA end in the "L" configuration. Thiolytic cleavage is catalyzed by the enzyme called thiolase.

12. The first reaction of fatty acid oxidation involves a set of enzymes know as acyl dehydrogenases. These are specific for fatty acids with long, medium, or short chains. The medium chain acyl dehydrogenase has been implicated in some instances of sudden infant death syndrome (SIDS).

13. The long chain acyl dehydrogenases are found in peroxisomes and this is where oxidation of long chain fatty acids (longer than 16 carbons) begins (not in the mitochondrial matrix). Oxidation here involves transfer of electrons to oxygen to make hydrogen peroxide, instead of $FADH_2$. Peroxisomal fatty acid oxidation is therefore LESS efficient than mitochondrial beta oxidation.

14. The first step of oxidation generates a *trans*-intermediate plus $FADH_2$. The second step involves addition of water across the *trans* double bond to create an intermediate with a hydroxyl on carbon 3 in the L configuration. The third step involves oxidation of the hydroxyl intermediate to a ketone on carbon 3. The last step involves cleaving off of an acetyl-CoA and production of a fatty acyl-CoA with two fewer carbons. The last step is catalyzed by the enzyme thiolase.

15. The reactions of beta oxidation up to the thiolase reaction chemically mirror the reactions of the oxidation of succinate up to oxaloacetate.

16. Seven cycles of beta oxidation of palmitoyl-CoA in the matrix yield 8 acetyl-CoAs.

17. Oxidation of biologically occurring fatty acids with *cis* double bonds requires two additional enzymes compared to oxidation of saturated fatty acids. These enzymes are enoyl-CoA-isomerase and 2,4-dienoyl-CoA-reductase.

Two computer nerds met in the thick
Of a programmers' summer picnic
He preferred a track pad
She used tablets like mad
Too bad that the two didn't click

18. Enoyl-CoA-isomerase converts *cis* bonds between carbons 3 and 4 to *trans* bonds between carbons 2 and 3. Since beta oxidation normally has *trans* bonded intermediates between carbons 2 and 3, this enzyme is sufficient for conversion of many naturally occurring fatty acids to be oxidized.

19. 2,4-dienoyl-CoA reductase acts on intermediates that have double bonds between carbons 2-3 and 4-5. It uses NADPH to reduce the two double bonds to one double bond and the resulting double bond is placed in a *cis* configuration between carbons 3-4. Enoyl-CoA-isomerase then can convert this intermediate to one with a *trans* double bond between carbons 2-3, thus allowing beta oxidation to continue.

Playing hookey one day sounded splendid
So no classes the truant attended
This excitement got trumped
After he bungie jumped
And discovered that he was suspended

20. Oxidation of fatty acids with odd numbers of carbons yields a final product of propionyl-CoA, not acetyl-CoA. Conversion of propionyl-CoA to succinyl-CoA requires an enzyme that uses a coenzyme group of vitamin B_{12}. This coenzyme contains a cobalt atom that provide a means of generating the radical necessary for the reaction.

21. Conversion of propionyl-CoA to succinyl-CoA requires three steps. The first is addition of a carboxyl-group to the middle carbon in the molecule. The overall process involves two unusual isomerizations and movement of a methyl group that utilizes the cobalt atom of vitamin B_{12}.

22. Ketone bodies are produced by the body when glucose precursors are not available to make glucose. Examples of ketone bodies include acetoacetate and hydroxybutyrate. Diabetics have problems with glucose metabolism and may produce ketone bodies to provide energy to keep the brain alive. One can detect this by the smell of acetone on their breath. The thiolase reaction in ketone body formation is the reversal of the same reaction that occurs in fatty acid beta oxidation.

23. Fatty acid biosynthesis occurs similarly to beta-oxidation, though in reverse. Important distinctions are noted below in a-f.

a. Fatty acid synthesis up to palmitate occurs in the cytoplasm, but beta oxidation occurs in mitochondrial matrix.

b. Fatty acids are built using an acyl carrier protein (ACP), but beta oxidation uses CoA.

c. NADPH is used to donate electrons in synthesis, but the NAD^+ and FAD are used to accept electrons in oxidation in the mitochondrion.

d. A three carbon molecule, malonyl-ACP donates two carbons to the growing fatty acid chain - a carbon dioxide is lost in the process. Beta oxidations yield two carbon acetyl-CoA units.

e. Synthesis of fatty acids longer than 16 carbons occurs in endoplasmic reticulum or mitochondrion. Oxidation of fatty acids longer than 16 carbons begins in peroxisomes.

f. In fatty acid biosynthesis, a D-hydroxyl intermediate is formed at carbon #3. In fatty acid oxidation, an L-hydroxyl intermediate is formed at carbon #3.

24. Acetyl-CoA carboxylase catalyzes the addition of a carboxyl group to acetyl-CoA to form malonyl-CoA.

25. The enzymes of fatty acid synthesis apart from acetyl-CoA carboxylase are contained in a complex known as fatty acid synthase. We will refer to the fatty acid sythase complex as the name of all of the enzyme for all of the reactions after acetyl-CoA carboxylase.

26. Fatty acid synthase produces the saturated 16 carbon fatty acid known as palmitate.

At the will reading late in the day
The executor stood up to say
“It feels kind of funny
Handing out all this money
As part of a dead giveaway”

27. Enzymes that produce unsaturation in fatty acid biosynthesis are called desaturases. Desaturases are found in the endoplasmic reticulum.

28. Essential fatty acids are those that must be provided in the diet of an organism, because the organism cannot synthesize them.

29. In mammals, linoleic and linolenic acids are essential fatty acids because these organisms cannot make double bonds closer to the end than the Δ-9 position (oleic acid is a Δ-9 fatty acid). Thus, linoleic acid (Δ 9,12 double bonds = omega 6 for an 18 carbon fatty acid) and linolenic acid (Δ 9,12,15 double bonds = omega 3 for an 18 carbon fatty acid) must be provided in the diet of mammals.

30. Fatty acids longer than 16 carbons are produced by action of enzymes called elongases. These are found in the endoplasmic reticulum and the mitochondrion.

31. Citrate acts as a shuttle to carry acetate to the cytoplasm from the mitochondrion when the citric acid cycle stops. Citrate lyase cleaves citrate in the cytoplasm to create oxaloacetate and acetyl-CoA.

32. Prostaglandins are hormone-like compounds made from arachidonic acid by action of an enzyme known as prostaglandin synthase. There are several prostaglandin synthases in the body. The reactions they catalyze are forming cyclic oxygen-containing compounds (that's what prostaglandins are), so the enzymes are also known as cyclooxygenases (or COX for short). The COX enzymes are known as COX-1 and COX-2.

33. Prostaglandins are involved in numerous physiological effects, including control of vasodilation/constriction, uterine contractions, aggregation/stickiness of platelets, inflammation/pain, and maintenance of stomach tissue, among others. Inhibitors of COX enzymes are called COX inhibitors. Aspirin and ibuprofen are non-steroidal drugs (called NSAIDs) that inhibit COX-1 and COX-2.

The ink drop expressed grumpily
Her wish to escape and be free
“I truly have been
Too long in the pen
This sentence is too long for me”

34. Prostaglandins produced by COX-2 enzymes appear to have no role in stomach maintenance, so inhibitors specific to them were sought. Examples include Celebrex and Vioxx, but they may have negative side effects on the heart.

35. Arachidonic acid is produced from linoleic acid released from glycerophospholipids by action of an enzyme known as phospholipase A_2 (PLA_2). PLA_2 can be inhibited by corticosteroids, so action of these compounds can also prevent prostaglandin formation indirectly. Corticosteroids are important for treatment of severe inflammation or pain.

36. Leukotrienes can also be produced from arachidonic acid. The pathway that leads to them does not involve cyclization, so that pathway is called the linear pathway to distinguish it from the cyclic pathway that leads to prostaglandins. Leukotrienes are involved in mucus production and bronchial constriction and play important roles in asthma attacks.

37. Another class of molecules made from prostaglandins is the thromboxanes. These molecules help to make platelets "sticky", favoring aggregation. Thus, taking aspirin reduces synthesis of prostaglandins, which in turn reduces amounts of thromboxanes, which reduces stickiness of platelets, which makes it harder for blood to clot. It is for this reason that people prone to clotting problems are sometimes advised to take aspirin daily.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Nucleotide Metabolism

1. Nucleotides consist of a) sugar, b) nitrogenous base, and c) phosphate.
2. Nucleosides consist of a a) sugar and b) nitrogenous base
3. The sugars of nucleosides and nucleotides are either ribose (found in ribonucleotides of RNA) or deoxyribose (found in deoxyribonucleotides of DNA).
4. The term nucleoside phosphate is equivalent to a nucleotide (nucleoside + phosphate + base = nucleotide). This is true whether it is a monophosphate, diphosphate, or triphosphate.
5. The nitrogenous bases found in nucleotides include adenine (purine), guanine (purine), thymine (pyrimidine), cytosine (pyrimidine), and uracil (pyrimidine).
6. The bases adenine, guanine, and cytosine are found in both ribonucleotides and deoxyribonucleotides. Thymine is almost always found in deoxyribonucleotides. Uracil is found primarily in ribonucleotides and rarely in DNA, but does appear as a deoxyribonucleotide intermediate in thymidine metabolism.
7. Nucleosides are named according to the base they contain. Nucleosides containing purines are named by adding "os" before the "ine." Thus, nucleosides containing guanine are called guanosine. Nucleosides containing pyrmidines are named with the suffix "idine" at the end of the name of the base they contain. Thus, the pyrimidine nucleosides are

The feudalist takes lots of notes
Everything from the baron he quotes
“When it comes to election
The final selection
Depends on how ev’ry Count votes”

‘Round the household it was overheard
Causing sadness that no one preferred
There’s no guarantees
For this terminal disease
The calendar’s days are numbered

cytidine, uridine, and thymidine.

8. Ribonucleotides are the building blocks of RNA and deoxyribonucleotides are the building blocks of DNA.

9. Nucleotides and nucleosides are made in cells by two general mechanisms - salvage pathways (use breakdown products of other nucleotides/nucleosides) or *de novo* pathways (synthesize nucleotides/nucleosides from scratch).

10. In salvage pathways, nucleic acids can be broken down to nucleoside monophosphates or individual bases. Monophosphates be rephosphorylated to triphosphates by kinases in order to reincorporate them into nucleic acids. Alternatively, nucleoside monophosphates can lose a phosphate (becoming nucleosides) or can lose the phosphate and the sugar to become a base. Bases can either be broken down or reconverted back to nucleoside monophosphate by addition of appropriate sugars and/or phosphates.

I confess that I truly can’t see
Solving problems mathematically
So don’t ask me then
How to double 2n
Cuz it all seems so 4n to me

11. *De novo* synthesis of nucleotides utilizes very simply precursors - amino acids, carbamoyl phosphate, and sugars. Activated carbons are donated by folate derivatives.

12. *De novo* synthesis of pyrimidines is fundamentally different from that of purines. In purine biosynthesis, the base is assembled on the sugar. In pyrimidine biosynthesis, the base is made apart from the sugar and later attached to it.

13. In *de novo* synthesis of nucleotides, ribonucleotides are synthesized first. Deoxyribonucleotides are made from ribonucleoside diphosphates. Atoms in the ring of pyrimidines come from aspartate and carbamoyl phosphate.

14. The enzyme carbamoyl phosphate synthetase has an interesting catalytic strategy involving channeling of the substrates through the enzyme as catalysis occurs. This is important because some of the intermediates (such as carboxyphosphate and carbamic acid) are very unstable in aqueous solution.

At the dance I was wanting for sure
To step out with a lass I prefer
With my leg in a cast
I couldn’t dance fast
But I sure had a crutch on her

15. The most important regulatory enzyme for the entire pathway of pyrimidine biosynthesis is aspartate transcarbamoylase (ATCase), which plays an important role in balancing the amounts of purines and pyrimidines and also measuring the amount of energy available (via the amount of ATP present). The enzyme catalyzes the linkage of aspartate to carbamoyl phosphate and is allosterically activated by ATP and

allosterically inactivated by CTP.

16. The first pyrimidine nucleotide made in the *de novo* pyrimidine pathway is UMP.

17. UMP is phosphorylated to UDP (by uridine monophosphate kinase) and then to UTP (by nucleoside diphosphokinase = NDPK) before conversion to CTP. Each nucleoside monophosphate has a specific kinase to convert it to the diphosphate, but all diphosphates (purines, pyrimidines, and all deoxyribonucleoside diphosphates) are converted to triphosphates by NDPK.

18. Conversion of UTP to CTP is catalyzed by the enzyme CTP synthase and it is inhibited by CTP, thus providing a balance between the amount of CTP and UTP.

19. *De novo* synthesis of purines uses atoms from aspartate, glycine, glutamine, carbon dioxide, and tetrahyrdofolate derivatives to make the purine ring.

20. The most important regulatory enzyme for the first part of purine biosynthesis is PRPP amidotransferase. It is inhibited fully by AMP and GMP, but is only partly inhibited (is still partly active) when only one of these molecules is present. This enzyme helps to control purine production and also slows purine production when one nucleotide gets to be in too high of a concentration.

21. In the process of making IMP, fumarate is released, thereby connecting purine biosynthesis to the citric acid cycle.

22. The first purine-like intermediate in *de novo* purine biosynthesis is inosinic acid (or IMP),

All American shoppers recall
Gazing out at the great urban sprawl
It really seems lame
Cuz they all look the same
See one center and you've seen a mall

which has the purine-like base hypoxanthine linked to ribose (and ribose is linked to phosphate).

23. IMP can be converted to AMP or GMP. The pathway by which IMP leads to GMP is inhibited by GMP and uses energy from ATP, whereas the pathway where IMP is converted to AMP is inhibited by AMP and uses energy from GTP. Thus, the critical balance of these two nucleotides is maintained using this scheme.

24. The pathway to GMP involves oxidation, whereas the pathway that leads to AMP uses aspartic acid to donate an amine and fumarate is again released.

25. Nucleoside monophosphates from *de novo* synthesis can be converted by kinases to nucleoside diphosphates and nucleoside triphosphates. These enzymes include AMP kinase (also called adenylate kinase) and GMP kinase (also called guanylate kinase), which convert nucleoside monophosphates to nucleoside diphosphates, and nucleoside diphosphate kinase (NDPK), which converts all nucleoside diphosphates to nucleoside triphosphates.

26. Ribonucleotide reductase (RNR) catalyzes the formation of deoxyribonucleotides from ribonucleotides. The substrates are ribonucleoside diphosphates (ADP, GDP, CDP, or UDP) and the products are deoxyribonucleoside diphosphates (dADP, dGDP, dCDP, or dUDP).

27. RNR has two pairs of two identical subunits - R_1 (large subunit) and R_2 (small subunit). R_1 has two allosteric binding sites and the active site of the enzyme. R_2 forms a tyrosine radical necessary for the reaction mechanism of the enzyme.

28. Ribonucleotide reductase is allosterically regulated via two binding sites - a specificity binding site (binds dNTPs and controls which substrates the enzyme binds

and which deoxyribonucleotides are made) and an activity binding site (controls whether or not enzyme is active - ATP activates, dATP inactivates).

29. Specificity sites act in a generally complementary fashion. Binding of deoxypyrimidine triphosphates to the specificity site tends to inhibit binding and reduction of pyrimidine diphosphates at the enzyme's active site and stimulates binding and reduction of purine diphosphates at the active site. Binding of deoxypurine triphosphates tends to inhibit reduction of purine diphosphates and stimulates reduction of pyrimidine diphosphates. Don't confuse the active site with the activity site. The ACTIVE SITE is where the reaction is catalyzed, whereas the ACTIVITY SITE is the allosteric binding site for ATP or dATP.

30. Synthesis of dTTP by the *de novo* pathway takes a convoluted pathway from dUDP to dUTP to dUMP. The latter reaction here is catalyzed dUTPase.

31. The *de novo* pathway for thymidine synthesis converts dUMP to dTMP, using a tetrahydrofolate derivative and the enyzme thymidylate synthase. In the process, dihydrofolate is produced and must be converted back to tetrahyrdolate in order to keep nucleotide synthesis occurring.

32. The enzyme involved in the conversion of dihydrofolate to tetrahydrofolate, dihydrofolate reductase (DHFR), is a target of anticancer drugs which inhibit the enzyme. An inhibitor of DHFR is methotrexate or aminopterin.

33. ATCase is regulated allosterically by ATP (activates) and CTP (inactivates). It is the most important regulatory enzyme in *de novo* pyrimidine biosynthesis and it helps to balance the relative amounts of purines and pyrimidines. Another important regulatory enzyme in the pathway is CTP synthase, which is inhibited by CTP. This enzyme helps balance the relative amounts of CTP and UTP.

MIRANDA
HELENA
BEATRICE
OPHELIA
JULIET
4
2
3
5
4

34. PRPP amidotransferase is an important regulatory enzyme for purine biosynthesis. It is inhibited by AMP, GMP, and IMP. If AMP is low and GMP is high (or vice-versa), the enzyme is reduced in activity, but still can function. This is important to help increase the amount of the other one, thus helping to balance AMP and GMP.

If you long for the beer drinking spaces
A brewery can be an oasis
Is a lager the tops
Because of its hops?
Decide on a case by case basis

35. Salvage of purine nucleotides is important metabolically - perhaps more so than salvage of pyrimidines. The enzyme HGPRT is involved in the direct salvage of guanine nucleotides and indirectly involved in salvage of adenine nucleotides through IMP and hypoxanthine.

36. Breakdown of purines results in production of xanthine. Oxidation of xanthine yields uric acid. This compound serves an excretory role in birds and dalmations (among other organisms). Uric acid is not very water soluble and can precipitate out in nerve cells, causing the painful condition known as gout. Gout often strikes in the big toe.

Mr. Ritz stood before all the backers
Thanking them for the six million smackers
It is rare when folks say
They rejoice every day
That you're gonna go even more crackers

37. Uric acid acts

as an antioxidant and may have protective roles against diseases, such as multiple sclerosis. The disease is successfully treated with allopurinol, which acts as a suicide inhibitor of the xanthine oxidase enzyme.

38. Severe combined immune deficiency arises from a deficiency of adenosine deaminase. In immune cells of patients with this disease, dATP accumulates, shutting off RNR and stopping cell division.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Nitrogen Metabolism

1. Reduction of nitrogen to a form useful for organisms (called nitrogen fixation) is a difficult and energetically costly process. Nitrogen fixation is made possible by bacteria that have an enzyme known as the nitrogenase complex.

2. In nitrogen fixation, N_2 is reduced to ammonium ion (NH_4^+). The process theoretically requires six electrons and 12 ATPs to make two ammonia molecules (NH_3), but hydrogen gas is always produced, so two additional electrons and 4 ATPs are also needed (giving a total of 8 electrons and 16 ATPs) .

3. Once reduced to ammonium ion, nitrogen can readily be incorporated into the amino acids glutamate, and glutamine.

4. Amino acids are grouped into families corresponding to the precursors they are made from. Students should know the amino acids that are simple transamination products - glutamate (made from alpha-ketoglutarate), glutamine (made from glutamate), aspartate (made from oxaloacetate), alanine (made from pyruvate), asparagine (made from aspartate).

5. Other reactions involving amino acids involve transfer of single carbons. This often involves derivatives of folic acid (folates). Note that folates can donate single carbons AND accept single carbons.

6. Humans require folates in their diet and do not synthesize them from scratch. Bacteria, on the other hand, make them from scratch. An intermediate they use in this regard is para-amino-benzoic acid (PABA). Folates exist in two main forms for our purposes - dihydrofolates and tetrahydrofolates.

The haters of music agree
It is not something they wish to see
From the sound of their talk
Whether Mozart or Bach
They hate violins on TV

7.Anticancer drugs and antibiotics sometimes target folates. Bacterial cells start synthesis of folates with para-amino benzoic acid and drugs that mimic this are the sulfa drugs. Humans get folate in their diet, so we are not susceptible to action of these drugs. Methotrexate is an anti-cancer drug that mimics folate.

8. Another molecule involved in single carbon reactions is known as S-Adenosyl-Methionine (SAM). It serves as a donor of methyl groups. After SAM donates its methyl group, it forms S-Adenylhomocysteine (SAH) that can be readily broken down to homocysteine. Elevated levels of homocysteine in the blood are associated with atherosclerosis. Reduction of homocysteine in the blood is accomplished with supplements of folic acid and, to a lesser extent, vitamins B_6 and B_{12}.

9. Essential amino acids for an organism are those amino acids that the organism cannot synthesize themselves and must be in their diet. Humans have 10 amino acids considered essential.

10. Breakdown of amino acids (catabolism) is divided into those amino acids whose carbon backbone forms intermediates in ketone body formation (acetoacetyl-CoA and acetyl-CoA = ketogenic), glucose metabolism (oxaloacetate or pyruvate = glucogenic), or both.

11. The Urea Cycle is involved in nitrogen metabolism in cells. Note how an amine group of aspartate is transferred to form urea. Students should know the names of the molecules of the pathway, as well as the pathway's involvement in making urea by splitting it off of arginine. Note also that the cycle occurs partly in the mitochondria and partly in the cytoplasm.

12. Uric acid and urea are excretory forms of nitrogen. Human primarily excrete urea, whereas birds (and dalmations) excrete uric acid. Uric acid, which is a breakdown product of guanine, is NOT very soluble in water and can form crystals, causing the disease known as gout.

Geriatrics are quick to address
A problem that causes distress
In turning the page
Of increasing one’s age
It’s not fun to feel so youthless

Jump to Chapter

1 / 2 / 3/ 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

DNA Replication, Repair, Recombination

1. DNA consists of a double helix. Each strand of the helix is a polymer of nucleotides joined together in phosphodiester linkages that have alternating sugar-phosphate-sugar-phosphate links. On the inside of the double helix are the complementary base pairs held together by hydrogen bonds.

2. The arrangement of the double helix is in an 'anti-parallel' fashion, meaning that one strand oriented in the 5' to 3' direction is directly paired to a complementary strand oriented in the 3' to 5' direction. Phosphodiester bonds involve linkage between the 5' phosphate group of the incoming nucleotide and the 3' hydroxyl of the previous nucleotide in the chain.

3. DNA contains four bases - A,T,C, and G arranged with A paired with T and G paired with C on the internal portion of the double helix. Hydrogen bonds stabilize these base pairs - two for the A-T pair and three for the G-C pair. Thus, G-C pairs are harder to break than A-T pairs.

4. DNA has a major and a minor groove arising from asymmetric glycosidic linkages between the deoxyribose sugar and each base in the double helix.

5. DNA has three major forms - A,B, and Z. The A and B forms are right-handed helices, whereas the Z form is a left-handed helix. The B form of DNA is the most prevalent one and contains about 10.5 bases per turn of the helix.

6. Z-DNA may have roles in marking the location of genes in eukaryotic chromosomes.

7. Another DNA form is the A form (actually discovered by Rosalind Franklin), which is more "compressed" and is also a right-handed helix. The A form is the form assumed by double strand RNA or RNA-DNA duplexes as well. RNA cannot exist in the B form due to steric hindrance arising from the oxygen on carbon number 2 of ribose, which is not present in the deoxyribose of DNA.

8. All DNA polymerases require a primer to start DNA synthesis. The primer is formed inside of cells by a special RNA polymerase known as primase. (RNA polymerase does not require a primer)

9. DNA replication proceeds by two distinct mechanisms (both 5'-3', however)- one on each strand. Leading strand and lagging strand synthesis occur by different mechanisms, but both are catalyzed by the same DNA replication complex (Pol III, in the case of *E. coli*).

10. Leading strand synthesis is continuous in the 5' to 3' direction. Lagging strand synthesis can only occur when the leading strand synthesis opens up a new single-stranded region for replication. The 5' to 3' syntheses on the lagging strand are discontinuous. The many pieces of lagging strand synthesis are called Okazaki fragments.

11. Okazaki fragments must be combined together ultimately. First, the RNA primer must be removed from each one. The 5'

to 3' exonuclease activity of DNA Polymerase I is needed to remove the initial RNA primer of leading strand synthesis, but is needed frequently to remove the primers of lagging strand synthesis.

The work of the dentist in serving
His patients was always unswerving
He got mighty praise
Working with the decays
But the root canals all were unnerving

12. DNA ligase is an enzyme that creates phosphodiester bonds between adjacent nucleotides between Okazaki fragments. Biotechnologists use this enzyme to join DNA fragments together to create recombinant molecules.

13. *E. coli* DNA replication occurs at 1000 base pairs per second. At 10 base pairs per turn, this represents a machine turning at 5000 to 6000 rpm. *E. coli*'s helicase protein (DNA B - part of the BC complex) unwinds DNA at a rate of at least 5000 - 6000 rpm. The protein separates strands ahead of the DNA Pol III so as to make single strands accessible for replication.

When the piracy expert exploded
All the support that he had was eroded
The cause of the storm?
He tried to stay warm
By wearing a jacket “down-loaded”

14. Unwinding of strands causes superhelical tension to increase ahead of the helicase. Topoisomerase II (gyrase) relieves the tension created by the helicase and is essential for replication to proceed efficiently.

15. DNA Polymerase III is very processive in its action, meaning that once it gets onto a DNA molecule, it stays on it for a long time replicating it. DNA Polymerase I is NOT very processive. The difference between the processivity properties of these two enzymes is because a protein called a sliding clamp (beta clamp in *E. coli*) holds DNA polymerase III to the DNA during replication, but it does not interact with DNA Polymerase I.

16. In *E. coli* DNA replication, a dimer of DNA Polymerase III is at the replication fork and performs most of the DNA replication in the cell. One portion of it replicates the leading strand and the other replicates the lagging strand. Leading strand synthesis is faster, so the lagging strand template sometimes loops out in a trombone-like fashion when the lagging strand replication falls behind.

17. Proteins at/near the replication fork and their functions include primase (makes RNA primers necessary for the DNA polymerase to act on), SSB (single stranded binding protein - protects single-stranded DNA and interacts with the replication proteins), DNA gyrase (topoisomerase II - relieves the superhelical tension created by helicase), Pol I (removes RNA primers), DNA ligase (joins DNA fragments together by

At Georgia Pacific the labor
Was performing an odd kind of caper
For the tiny and svelte
They made tissue belts
Out of something they dubbed the waist paper

catalyzing synthesis of phosphodiester bonds at nick sites), and helicase (unwinds double helix).

18. DNA Polymerase I is much more abundant in cells than DNA Polymerase III and it functions to remove RNA primers on Okazaki fragments and fill in the short regions where the RNA bases were with DNA.

19. DNA polymerase I has three enzymatic activities - a 5' to 3' DNA polymerase activity, a 3' to 5' exonuclease activity (also called proofreading), and a 5' to 3' exonuclease activity.

20. All DNA polymerases require a primer to start DNA synthesis. The primer is formed inside of cells by a special RNA polymerase known as primase. (All RNA polymerases do not require a primer)

21. Removal of RNA primers in *E. coli* requires DNA Polymerase I's 5' to 3' activity. Without it, the cell will die.

22. The linking number (L) of a DNA is the sum of the number of twists (T) of a DNA plus the number of writhes (W). Thus, L = T + W. The twists are the number of times two the two helices cross each other. The writhe is the number of superhelical turns found in a DNA. Writhes can be positive or negative and in either case, when the W is a non-zero value, the molecule is said to be superhelical or to have superhelicity.

The vegetarian editor takes
Very long evening mealtime breaks
And wears a big frown
Every time she sits down
Perhaps it's because of missed steaks

23. Writhing of DNA occurs in an attempt by a DNA molecule to "relax." A DNA molecule is relaxed when its number of base pairs (bp) per twist (T) is that of B-DNA (10.4-10.5 bp per turn). Thus, if one takes a relaxed circular DNA, opens it and removes two twists from it and then closes it, the number of twists will

decrease, but the number of base pairs remains the same. In this case, the numbers of bp per twist will INCREASE. This causes a tension that is relieved by the DNA TWISTING two turns. This will cause the writhe to compensate by forming two negative superhelical turns, giving W a value of negative two. Note that the linking number remains the same.

Old Donald the Disneyland gent
Hurt his foot when it banged the cement
Still he danced with much pain
And he never complained
To set a lame duck precedent

24. On the other hand if one takes a relaxed circular DNA, opens it and adds twists to it and then closes it, the number of twists will increase, but the number of base pairs remains the same. In this case, the numbers of bp per twist will DECREASE. The DNA will relax by UNTWISTING two turns, which will cause the writhe increase to a value of positive two.

The cereal farmer did cry
Of genetical problems – here's why
His crop it appeared
Was re-engineered
So all of his plants turned a-rye

25. Initiation of replication in *E. coli* occurs at a specific site on the *E. coli* genomic DNA, known as OriC, in the cell's circular chromosome. The OriC site contains three repeats of an AT rich sequence near some sequences bound by the DNA A protein.

26. Replication initiation begins with binding of the several copies of the dnaA protein to the OriC site. Bending and wrapping of the DNA around dnaA proteins causes the AT-rich sequences noted above to become single-stranded.

27. Next, the dnaBC complex binds the dnaB protein (helicase) to each of the single strands in opposite orientations. The dnaC protein is released in the process.

28. Next, SSB and primase bind the exposed single-stranded regions and cause DNA A protein to be released. The primases begin synthesizing RNA primers (remember - 5' to 3' RNA synthesis only also) in opposite directions on each strand. The primases DO NOT require a pre-existing primer to function.

29. Note that replication of the *E. coli* circular DNA is bi-directional - two replication forks pointed in opposite directions from the origin. They meet later at a termination site on the other side of the genomic DNA.

30. Eukaryotic DNA replication is coordinated tightly with the cell cycle. Checkpoints during the cell cycle ensure that progression through the cell cycle does not occur if there are problems with the DNA.

31. When such conditions arise, the repair process can be initiated and if repair cannot be performed, a series of events resulting in cellular death may start to occur.

32. Eukaryotic chromosomes differ from prokaryotic DNAs in being linear. The linear ends of the chromosomes are called

telomeres. Telomeric sequences have thousands of copies of repeats of short sequences.

33. The enzyme that builds telomeres is called telomerase and is found predominantly in fetal and cancer cells, as well as fertilized eggs. Differentiated cells for the most part do not appear to have an active telomerase.

34. With each round of DNA replication, linear chromosomes in eukaryotes shorten. Thus, the longer the telomeric sequences are in a chromosome, the more divisions it can undergo before the telomeres are "eaten up".

35. Telomerase is a reverse transcriptase - an enzyme that uses an RNA template (a circular RNA that it carries) to synthesize DNA. Other reverse transcriptases are found in retroviruses, such as HIV.

36. Damage to DNA can occur chemically (deamination of adenine to form hypoxanthine), by oxidation (creation of 8-oxo-guanine by reactive oxygen species reaction), by reaction with an aflatoxin metabolite, by reaction with a cross-linking reagent, such as psoralen, and by dimerization of adjacent thymines stimulated by ultraviolet light.

37. These systems require repair - described below. Another system requiring repair is DNA sliding, which can occur amid repeating sequences. Lack of a repair system for these leads to Huntington's disease.

38. DNA Repair systems to repair damage include

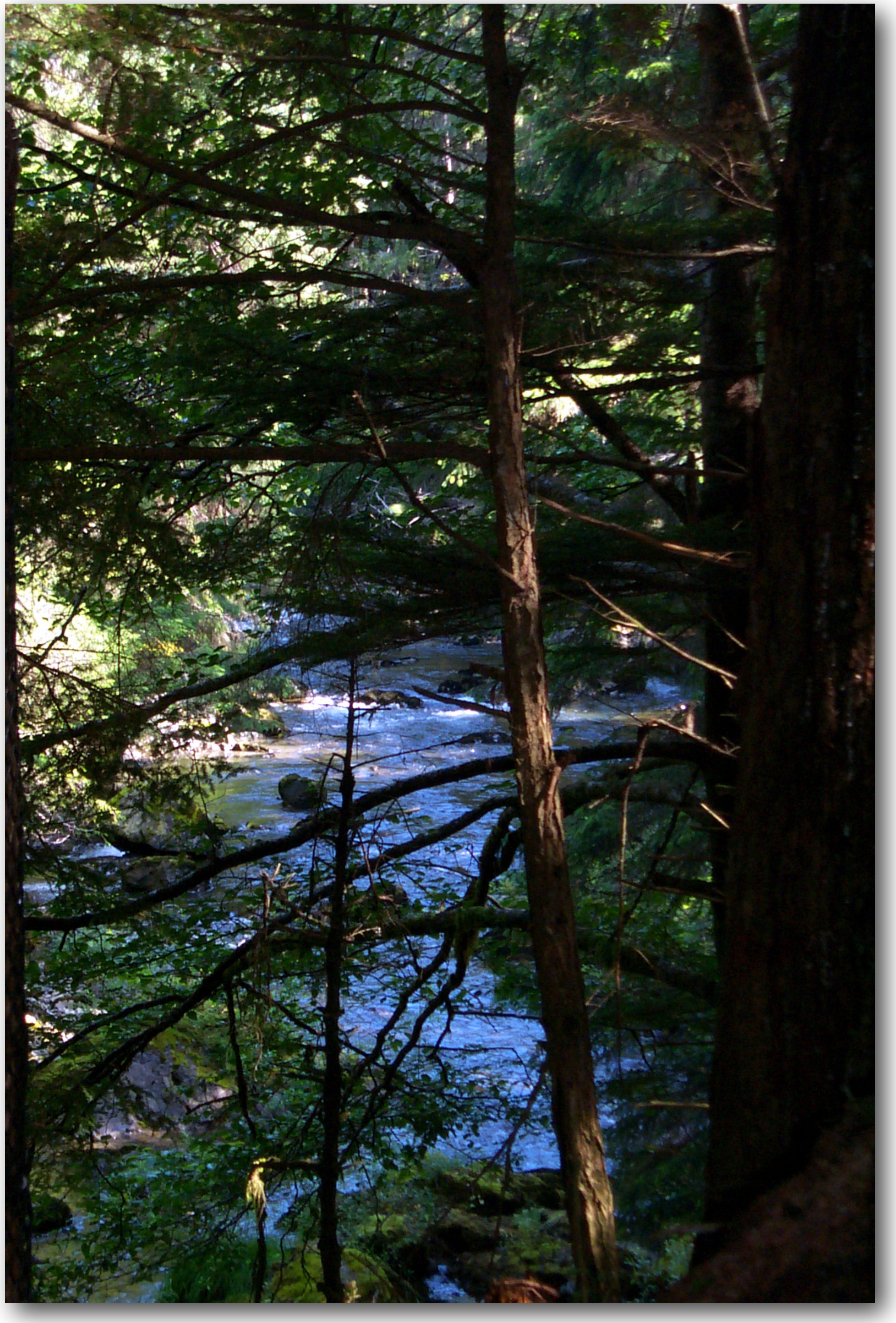
A. Proofreading (technically only repairs mismatches, not damage) was discussed earlier. It involves 3'-5' exonucleolytic activity of a DNA polymerase and it occurs as DNA is being replicated.

B. Mismatch repair - fixing of mismatches that occur, largely as a result of errors in replication.

C. Nucleotide Excision Repair - Excision of a group of nucleotides followed by replacement with correct ones by DNA polymerase. Removal of thymine dimers, for example

D. Base excision repair - Excision of a damaged base - for example uracil glycosylase removal of U from DNA.

39. In *E. coli*, mismatch repair occurs as a result of action of the proteins MutS (recognizes mismatch), MutL (recruits MutH), and MutH (nicks newly synthesized strand of DNA to allow exonucleolytic removal of nucleotides around the mismatch).



40. Nucleotide excision repair can be used to repair thymine dimers. Here, a segment of DNA containing the damage is removed by uvrABC excinuclease. The gap is then filled in using DNA Polymerase I and DNA ligase.

41. Base excision repair can removed damaged based from DNA. It differs from nucleotide excision repair in removing the damaged base first, followed by removal of a segment where the base was.

42. Disruption of error correction systems can have severe consequences.

43. Error-related systems associated with cancer include HNPCC (colon cancer) and BRC-A, which is involved in DNA repair. A critical protein for monitoring DNA for damage prior to division is p53. It can stop the cell cycle if it senses damage and initiate repair. If repair is unable to be performed, p53 can induce cellular suicide - apoptosis.

44. An Ames test uses a selectable marker that can give a readily observable phenotype (such as growth on antibiotic) when mutation

happens. By comparing the number of cells with the observable phenotype in a the presence of a test compound to the number of cells in another tube lacking that compound, the mutagenicity of a compound can be determined.

45. Recombination of DNA results in mixing and matching of DNA sequences. The process occurs most often between homologous sequences on different chromosomes. The process can be quite active during meiosis.

46. Recombination proceeds through formation of a Holliday junction. Holliday junctions form as a result of alignment of homologous sequences, followed by cleavage of strands on each chromosome, invasion of the strands into the opposite chromosome, movement of the junction, another cleavage reaction, followed by reformation of phosphodiester bonds.

47. Enzymes involved in recombination are called recombinases and are similar in function to the integrase of HIV.

I'm wondering what I should do
For my dentist who's feeling quite blue
Her work with decays
Has caused a malaise
What is more, she has got fillings too

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Transcription

1. Transcription is the process where RNA is made using DNA as a template. Students should ABSOLUTELY not mix up or misuse the terms DNA Replication, Transcription, and Translation.

2. RNA polymerization requires an enzyme called RNA polymerase. It can start a chain without a primer, incorporates nucleotides into a growing chain in the 5' to 3' direction using phosphodiester bonds, and uses ATP, GTP, CTP, and UTP as starting compounds. The product of RNA polymerization is called a transcript.

3. The 5' -most nucleotide in RNA has three phosphates on it. All other nucleotides in RNA have only the single phosphate of a phosphodiester bond. Synthesis of the phosphodiester bond arises from nucleophilic attack of the 3' oxygen on the internal phosphate (closest to carbon 5 of the ribose) of the incoming 5' nucleotide.

Perhaps he was being perverse
Using mirrors while writing so terse
Wouldn’t you know it
This most backwards poet
Did all of his writing inverse

4. Cells have three main types of RNA - mRNA (carries message to be translated into protein), tRNA (carries amino acids to ribosomes for incorporation into protein), and rRNA (components of ribosomes). Small RNAs are a fourth type of RNA that play important roles in control gene expression.

5. In *E. coli*, all of the RNAs are made by a single polymerase, known as RNA Polymerase. Eukaryotic cells have three RNA polymerases - RNA Polymerase I (rRNAs), RNA Polymerase II (mRNAs and snRNAs), and RNA Polymerase III (tRNAs).

Perhaps it was heavenly love
Or coincidences thereof
That gave Matt a ‘B’
In trigonometry
He thinks ‘twas a sine from above

6. *E. coli* RNA Polymerase has five distinct polypeptide subunits we discussed in class - alpha, beta, beta prime, and sigma.

7. Footprinting is a technique for determining where on a DNA molecule a protein is bound.

8. Promoters in *E. coli* (RNA polymerase binding sites adjacent to genes) function with widely varying rates of efficiency - some stimulating initiation of transcription every few seconds, others only one or twice per life cycle. One way for a promoter to control such events is via variation in conserved sequences. *E. coli* genes have two conserved sequences - one at -10 relative to the transcription start site (TATAAT) and another at -35 relative to the transcription start site (TTGACA).

9. The more closely a given promoter's sequence matches the consensus sequence of the -10 sequence, the more active the promoter is at initiating transcription. RNA Polymerase slides

along the DNA and when the sigma subunit identifies a promoter site, it stops.

10. Different sigma factors (such as the one made during heat shock) allow the cell to turn on sets of genes with different sequences in the -10/-35 sequences as needs arise.

11. Transcription occurs in a DNA "bubble" in which the RNA is partly base paired to the template strand, with the 5' end of the RNA "hanging" off. The bubble moves as the RNA polymerase moves. Ahead of the bubble, positive supercoils are created. This superhelical tension is relieved by DNA Gyrase. As the strands come back together behind the bubble, negative supercoiling occurs, and this is relieved by a topoisomerase.

12. The strand of DNA being copied is known as the template strand. It runs 3' to 5', since the RNA is being made 5' to 3' complementary to it.

13. The strand of DNA that is NOT copied in transcription is called the coding strand, since it has the same sequence as the RNA except for having T instead of U.

14. Transcription occurs in three phases - initiation, elongation, and termination. The initiation phase creates a open transcript complex and this phase can be inhibited in prokaryotes by rifampicin. During the initiation phase, the transcription bubble is formed and the first ten or so nucleotides are polymerized together by RNA polymerase. During the initiation phase, the sigma factor is released from RNA polymerase.

15. Transcription occurs under the 'control' of promoters. The more strongly RNA Polymerase (in prokaryotes) binds to the promoter, the more transcripts are made of that particular gene. The more transcripts are made, the promoter is referred to as being 'stronger.'

16. Transcription termination occurs by both factor dependent means

Henry Ford wins the prize, but of course
For breaking a rule some endorse
His particular zeal
For the automobile
Made him put his cars ‘fore the horse

(requires a protein factor) and factor independent means (no extra protein required). The factor independent method relies on formation of a duplex sequence of GC base pairs immediately ahead of a stretch of U's. The duplex destabilizes the RNA-DNA duplex and this is favored by the relatively weak hydrogen bonds of the U-A interactions.

17. The factor involved in factor dependent transcription termination in *E. coli* is called rho. It binds to the 5' end of an RNA being made and (using ATP energy) "climbs" the RNA until it reaches the RNA polymerase. There it destabilizes the RNA/DNA duplex, favoring the release of the RNA polymerase from the DNA and the RNA from the DNA, as well.

18. In prokaryotes, tRNAs are the most altered (processed) RNAs. Modifications start with their being cleaved from a larger RNA containing both tRNAs and rRNAs. Ribonuclease P is a ribozyme (catalytic RNA) that cleaves the 5' end of tRNAs from the larger RNA. Ribonuclease III catalyzes excision of rRNAs from the larger molecule.

19. Eukaryotes and prokaryotes differ significantly in the relationship between transcription and translation. Prokaryotes have no nucleus. In them, translation starts oftentimes WHILE a message is being transcribed. There are no significant modifications to mRNAs in prokaryotes.

20. In eukaryotes, transcription and translation are spacially separated. Transcription occurs in the nucleus, whereas translation occurs in the cytoplasm. In addition, eukaryotic mRNAs are modified at the 5' end (capping), the 3' end (polyadenylation) and even in the middle (editing and splicing).

21. Eukaryotes have 3 specialized RNA polymerases. They differ in their sensitivity to alpha-amanitin (a poison from some mushrooms). RNA polymerase II (makes mRNAs) is the most sensitive. RNA polymerase III (makes tRNAs and small rRNA) has moderate sensitivity and RNA polymerase I (makes large rRNAs) has low sensitivity.

22. Sequence elements affect transcription of eukaryotic genes. They include the TATA box (positioned approximately -30 to -100), and a CAAT box and GC box (-40 to -150).

23. The TATA box is not found in front of all eukaryotic genes, but is essential for strong transcription.

24. The promoters for each RNA polymerase are different in structure.

25. Enhancer sequence elements are DNA sequences that are bound by enhancer (transcription factor) proteins. Enhancer proteins act in this way to enhance transcription of genes located up to many thousands of base pairs upstream (ahead of), downstream (down from) or even in the middle of genes.

The geology students bemoan
Each exam has the same overtone
The grading is picky
And the questions are tricky
As if they were written in stone

26. RNA Polymerase II in eukaryotes differs from RNA polymerase in *E. coli* in not binding to the DNA directly, but rather, it must bind to another protein that binds to the promoter first.

27. Transcription factors assist RNA Polymerase II in binding. For genes with a TATA box, the transcription factor TFIID binds first. It contains a subunit called TBP (TATA-Binding Protein) that

When mom told him what would displease her
Little Brutus thought her an old geezer
When a chance would arise
He would spurn her advice
And so started running with Caesars

recognizes and binds to the TATA sequence.

28. In eukaryotes, rRNA is made by the action of RNA Polymerase I. It is made as a precursor and then processed, not unlike what occurs in prokaryotes.

29. tRNAs in eukaryotes are made by action of RNA Polymerase III. Processing of them includes removal of a 5' leader sequence and part of a 3' tail, as well as addition of a CCA sequence at the new 3' end. The amino acid gets attached to the 3' end of the tRNA.

My wife said last night I did keep
Telling stories when counting the sheep
I said many things
From Lord of the Rings
'Cuz I was Tolkien in my sleep

30. In contrast to prokaryotic mRNAs, eukaryotic mRNAs are extensively modified. Modifications include

   a. Addition of a 5'-5' cap of a methyl guanosine to protect from degradation
   b. Addition of a poly-A tail at the 3' end under the control of the sequence AAUAAA near the end of the mRNA (also to protect from degradation)
   c. Editing - modification of bases chemically, such as was described in class for the Apo-B proteins
   d. Splicing - removal of introns between exons.

31. Splicing is a modification to eukaryotic RNAs that occurs in the middle of the RNA. Splicing occurs to mRNAs, tRNAs and rRNAs in eukaryotes.

32. Splicing involves removal of internal sequences from RNA followed by joining of ends. The removed sequences are called introns. The segments that make it into the final RNA are called exons.

33. The only sequences common to all spliced RNAs are a GU sequence at the 5' end of the intron and an AG at the 3' end of the intron. A third sequence - an A residue surrounded by pyrimidines also is common.

34. Protein/RNA complexes called snRNPs mediate the splicing process in higher eukaryotes. snRNPs contain small nuclear RNAs (snRNAs) and proteins.

35. In splicing, the hydroxyl of the A residue attacks the phosphate of the phosphodiester bond at the 5' end of the intron, creating a 5'-2' bond (part of the lariat structure). Attack by the released 3' end of the exon on the 3' end of the intron joins the two exon ends and releases the intron as a lariat.

Everywhere now it seems it's the rage
Spending money as if to upstage
The neighbors next door
Who spend even more
These people should act more their wage

36. Exon shuffling (occurs in splicing in

different tissues) allows cells to make many versions of protein from a single sequence. This is important in immunology and in fine-tuning cellular needs that are tissue specific.

37. Lower eukaryotes are able to excise introns by an autocatalytic mechanism. At least one prokaryotic gene is spliced autocatalytically.

38. In splicing, the U_1 snRNA forms base pairing with the 5' end of the intron sequence.

39. In splicing, the U_2 snRNA froms base pairs with the pyrimidine-rich region in the intron and with the snRNA of U_6. Pairing with the intron forces outwards the 'A' residue that attacks the phosphate, as noted in class.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Translation

1. Translation (protein synthesis) is performed by ribosomes on mRNA and occurs in the 5' to 3' direction along the mRNA.
2. The rate of translation in bacteria is about the same as the rate of transcription (45-50 bases or 15-17 amino acids per second). The 5' end of the coding region corresponds to the amino end of the protein. The 3' terminus of the coding region corresponds to the carboxyl end of the protein.
3. Translation is performed by ribosomes on mRNA and occurs in the 5' to 3' direction. The rate of translation in bacteria is about the same as the rate of transcription (45-60 bases or 15-20 amino acids per second).
4. The 5' end of the coding region corresponds to the amino end of the protein. The 3' terminus of the coding region corresponds to the carboxyl end of the protein.
5. As polypeptides are being synthesized, the previously synthesized chain is attached to the free amine of the incoming (new) amino acid and the entire complex is, as a result, attached to the 'new' tRNA. Thus, polypeptides are synthesized in the amino to carboxyl terminus.

The teenager’s friends were all crowing
Of the show to which they were a-going
Jan worried that she
Would be absentee
Because she had parents all no-ing

6. Transcription and translation are coupled together in bacteria, but not in eukaryotes.
7. Translational accuracy is about one error per thousand to ten thousand amino acids. Greater accuracy would slow translation

down, so a balance is struck between the need for accuracy and the need to synthesize proteins reasonably rapidly.

8. tRNAs have extensive, but short self-complementary regions. tRNAs themselves are typically 73-93 bases long, with duplex regions at least partly in the A form.

9. The shape of tRNAs is that of an 'L'. At the 5' end of the tRNA is usually a 'G' and at the 3' end is a CCA.

10. Enzymes that catalyze the linkage of amino acids to tRNA's 3' or 2' ends are called aminoacyl-tRNA-synthetases. There are 20 of these enzymes - one for each amino acid.

11. Amino acids are linked to tRNAs by ester bonds between the carboxyl group of the amino acid and either the 2' or 3' hydroxyl of the ribose of the terminal adenosine residue of the tRNA.

The hypnotist took up his station
With a dentist to ease the sensation
The number one question
Will the power of suggestion
Let a trance end dental medication?

12. The anticodon loop has three bases complementary to the codon in the mRNA. tRNAs provide the translation function between nucleic acid sequence and amino acids. The anticodon loop frequently contains the inosine base. The base at the 3' end of the codon of the mRNA (corresponds to the base at the 5' end of the anticodon in the tRNA) is called the wobble base because it is less important for specifying the amino acid to be inserted than the first two bases.

An ornithological gent
Had his day truly filled with lament
To make a new sweater
He required some goose feathers
But there was a large down payMENT

The heir didn't think it was great
With his uncle now at heaven's gate
He got zero stocks
Just a hundred old clocks
So he's now winding up his estate

13. Aminoacyl-tRNA synthetases have the ability to recognize and correct errors in joining of amino acids to tRNAs. For example, if one puts the wrong amino acid on the end of a tRNA and then adds an appropriate aminoacyl-tRNA synthetase, the amino acid is readily removed.

14. Two regions of aminoacyl-tRNA synthetases are important for editing - called the activation site and the editing site.

15. There are two classes of amino acid tRNA synthetases. They differ in the way they bind tRNAs and in which hydroxyl of the ribose ring they attach the amino acid to.

16. Class I amino acid tRNA synthetases attach the amino acid to the hydroxyl on carbon #2. Class II enzymes attach the amino acid to the hydroxyl on carbon #3.

17. Base pairings in RNA are slightly different than in DNA. For example, G-U base pairs are not unstable. "I" (inosine) can also pair with C,U, or A.

18. In the genetic code, there are 64 possible combinations of the bases of the codon. Three of the possibilities (UAA, UGA, and UAG) are used as 'stop' codons. They tell the ribosomes where to stop making protein. A start codon is AUG and it codes for methionine. Since there are 61 codons used to code for amino acids and there are 20 amino acids, there is therefore 'redundancy' in the genetic code.

19. The Shine-Dalgarno sequence (GGAGG) is located near the AUG start codon in prokaryotic sequences. It is complementary to a sequence in the 16S rRNA and serves to help align the ribosome with the start site for translation in prokaryotes.

20. In prokaryotes, the first amino acid incorporated into a protein is a formylated form of methionine called fMet. The formyl group is put onto methionine after it is in the tRNA by a transformylase enzyme.

21. Formylation of methionine in prokaryotes protects the otherwise free amino end from reacting intramolecularly and terminating transcription.

22. Peptides exit the ribosome as they are being synthesized via a tunnel in the structure.

23. Ribosomes have three sites for binding/holding/releasing tRNAs. They are called the A,P, and E sites, corresponding to the order in which tRNAs move through them (except for the very first one known as the initiator tRNA = Met-tRNA^f).

24. Initiation of protein synthesis starts with binding of IF$_1$ and IF$_3$ to the 30S ribosomal unit.

25. IF$_2$ (when bound to GTP) acts to carry the Met-tRNA^f to the P site of the 30S subunit and base pairs it with the AUG start codon. IF$_3$ departs

in the process. The complex of mRNA, IF$_1$, IF$_2$, and Met-tRNA^f is called the 30S initiation complex.

26. Hydrolysis of the GTP in IF$_2$ results in release of the IF$_2$ and IF$_1$ from the initiation complex. That, coupled with binding of the 50S subunit yields the 70S initiation complex with Met-tRNA^f in the P site and the A and E sites open.

27. The process of elongation begins on the 70S initiation complex. EF-Tu (a G protein coupled to GTP) carries a charged tRNA to the A site of the complex. If the tRNA anti-codon base pairs properly with the codon in the mRNA, it stays matched with the codon and GTP is hydrolyzed on EF-Tu and EF-TuGDP is released. If the tRNA anti-codon does not form a stable base pairing with the complex, the entire charged tRNA-EF-Tu-GTP complex dissociates.

28. Next, the peptide group on the tRNA in the P site is transferred and covalently linked via peptide bond to the amino acid on the tRNA in the A site. This reaction is catalyzed by an enzymatic activity called peptidyltransferase - a ribozyme activity of the 23S rRNA in the 50S subunit.

29. The tRNA in the A site along with the peptide it is covalently attached to is transferred to the P site as the "empty" tRNA in the P site is moved to the E site. EF-G-GTP is involved in the process and GTP is hydrolyzed in the process. EF-G-GTP has a similarity to the tRNAaminoacid-EF-Tu-GTP complex and may act to displace it.

30. As the old tRNA is released from the E site, the

The Dominoes patrons perchance
Had good reason for looking askance
When a big no-no
Came from Yoko Ono
Who asked them to give pizza chants

At the meteorologists' fair
Each attendee was made most aware
Though they always want surety
There's no job security
Since their future is up in the air

empty A site accepts the aminoacyl tRNA corresponding to the next codon. The net result of one turn of this cycle is that the polypeptide has grown by one amino acid residue and the ribosome has moved along the mRNA by three nucleotide residues. The process is repeated until a termination signal is reached.

31. The process of translation termination begins when a stop codon appears in the A site of the ribosome. Termination of translation requires action of release factors (RF_1, RF_2).

32. RF_1 and RF_2 carry water to the A site. The peptidyl transferase transfers the polypeptide on the tRNA in the P site to water, thus releasing the completed polypeptide from the ribosome.

The farmer just lost a big frown
Because his lost cow has been found
When asked to define
How he got his bovine
He said that he just tractor down

33. Eukaryotic translation is mechanistically similar to prokaryotic translation. Differences include ribosomes (40S vs 30S and 60S vs 50S), rRNAs (28S, 18S, 5.8S, and 5S), mRNAs (cap at 5' end and polyA at 3' end, both involved in translation), and lack of formyl group on initiator tRNA's methionine.

34. Other translational differences in eukaryotes relate to the structure of eukaryotic cells. Targeting of proteins to organelles, the membrane, or outside of the cell, starts during protein synthesis. Proteins destined to leave the cytoplasm have a signal sequence, consisting of a stretch of hydrophobic amino acids near their amino terminus.

35. When the signal sequence emerges from the ribosome during translation, it is recognized by the signal recognition particle (SRP), which takes the entire ribosome/mRNA/polypeptide complex to the endoplasmic reticulum. There it interacts with the SRP receptor and in the process it links the ribosome with the emerging polypeptide sequence to the translocon.

36. The polypeptide passes through the translocon channel as it is being made and when the signal sequence completely exits the translocon, a signal peptidase clips it free of the rest of the polypeptide. The translation process continues until the stop codon is reached and then everything releases from the translocon.

37. The polypeptide remains in the endoplasmic reticulum where it is further process to travel to the Golgi for additional processing and targeting. Other sequences in the polypeptide may help to direct modifications and/or the final destination of

For a child acting out it's transparent
They're rewarded for acting aberrant
If you question the cause
Of the child's many flaws
The answer's most certainly apparent

the mature protein.

38. Antibiotics frequently are designed to target various aspects of translation. They include streptomycin (interferes with binding of formylmethionyl tRNA to ribosome), tetracytline (inhibits binding of aminoacy-tRNAs), chloramphenical (inhibits peptidyl transferase activity), and puromycin (causes premature chain termination).

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Key Points - Gene Expression

1. Gene expression refers to the processes that result in the production of functional protein. Gene expression can be controlled at the levels of transcription, processing (splicing in eukaryotes), translation, mRNA stability, and protein stability. Tissue-specific gene expression is essential for multicellular, differentiated organisms.

2. Transcription factors, as noted previously, are proteins that bind to DNA and affect the transcription of genes located near where they bind. Common DNA-binding structures are found in the diverse set of transcription factors that are know. They include motifs (motifs - structural features) for helix-turn-helix, homeodomains, leucine zippers, and zinc fingers.

3. Leucine zipper structures are found in adjacent alpha helices and contain regions with leucine residues appearing about every 7 amino acids. The leucine interact with each other to hold the strands together and in doing so allow other portions of the helix to bind DNA properly.

4.Zinc fingers are structures with cysteine residues that hold zinc ions and create a finger-like structure that can stick into the DNA helix.

5. Proteins that bind to specific DNA sequences must "read" the sequence of bases inside the helix, usually by inserting a portion of themselves into a groove of the DNA and assessing the hydrogen bonding molecules inside. Since different base pairs have unique hydrogen bonding orientations, the proteins find and bind to specific base sequences.

6. Control of gene expression is

I just got mad and I cursed
When I learned that my lunch plans reversed
Someone buried my brat
Underground in a spot
And so I exhumed all the wurst

also essential for prokaryotic organisms to be able to respond properly to their environments. For example, *E. coli* prefers glucose for energy, but must be able to use other sugars, like lactose, when they are available.

7. An operon is a prokaryotic system for organizing genes all under the same transcriptional control. Genes on the same operon in prokaryotes are all synthesized on the same mRNA. mRNAs containing multiple gene coding sequences are referred to as polycistronic.

8. The lactose operon consists of three linked structural genes that encode enzymes of lactose utilization, plus adjacent regulatory sites. The three enzymes --z, y, and a--encode beta-galactosidase, beta-galactoside permease (a transport protein), and thiogalactoside transacetylase (an enzyme of still unclear metabolic function), respectively.

9. Transcription of the lac operon commences at a promoter (lacP) before lacZ and transcribes a 5,200 nucleotide messenger RNA molecule (mRNA), ending at a terminator beyond lacA.

10. X-Gal is a synthetic substance used to study lac operon expression. X-Gal has the useful property that it turns blue when acted on by beta-galactosidase, giving a measure of how much the operon has been induced by the amount of blue color produced.

11. Negative transcriptional regulation of the lac operon is accomplished by a protein known as the lac repressor. It binds the operon's operator region and inhibits transcription.

12. In the absence of inducer molecules, the lac repressor tightly binds to the operator and inhibits transcription of the operon. When inducer molecules are present, they bind to the lac repressor and change its shape and reduce its ability to bind the operator, thus allowing the RNA polymerase to bind the promoter and start transcription.

13. The promoter sequence of the lac operon differs somewhat from the ideal consensus sequence of an *E. coli* promoter. Consequently, in the absence of positive acting elements, the lac promoter does not function well on its own. A protein that acts positively to help activate the lac operon is the CRP (cAMP Receptor Protein).

14. CRP (also called CAP) must bind to cAMP in order to function. When CRP binds cAMP, its affinity increases for the lac operon adjacent to the RNA polymerase binding site (-68 to -55). This binding facilitates

Master gardeners think it is wise
So listen when these folks advise
There’s no number one
With just a green thumb
You also need two fertile eyes

transcription of the lac operon by stimulating the binding of RNA polymerase to begin transcription.

15. When both CAP and the lac repressor are bound to the lac operon, the repressor 'wins', shutting down transcription of the operon.

16. In eukaryotic cells, DNA is wrapped up (coiled) with basic proteins called histones. Histone sequences are strongly conserved from yeast to humans.

17. Four histones form a core around which DNA is wrapped. This core contains two copies each of histones H2A, H2B, H3, and H4. This core of proteins is called an octamer.

18. The appearance of chromatin DNA is that of beads on a string, with the octamer wrapped with DNA composing the beads and the DNA strand coated with histone H1 (and H5) composing the string.

19. Histones of the octamer have strong structural similarity to each other.

20. Wrapping of DNA around the histone octamer provides only partial compression of the length of a DNA molecule. Additional compression occurs as a result of coiling of octamer/DNA complexes as well, forming higher order structures.

21. Enhancer sequences (also called sequence elements) are bound by enhancer proteins and are found only in eukaryotes. Multiple enhance sequences may be present before the start site of a particular gene. Binding of enhancer proteins to enhancer sequences allows for tissue specific expression of genes if the enhancer proteins themselves are expressed tissue specifically. Enhancer proteins help to "clear" out the histones from a region of a chromosome to allow transcription to occur.

The Antarctic fishermen still
Report that they quite get a thrill
Their common response?
They feel like James Bonds
Cuz they've got a license to krill

22. Nuclear hormone receptors, such as the estrogen receptor, have DNA binding domains and ligand binding domains. The binding of the estradiol (and estrogen) ligand to the estrogen receptor causes a conformational change in the protein, but does not change the binding of the protein to DNA. Binding of the estradiol DOES appear to activate the protein and thus activate transcription of the genes that the receptor binds to the promoter of.

23. The key to action of the nuclear hormone receptor that binds estradiol is that binding of estradiol favors binding of the receptor to co-activator proteins. These co-activator proteins help to turn on transcription of the relevant genes. Binding of co-activator proteins by transcriptional factors, such as the estrogen receptor is called recruitment.

As the photon took off for his flight
He packed up his things really tight
When asked why he dragged
Only one Duffel bag
He said he was traveling light

24. An antagonist of the estrogen receptor is the drug tamoxifen. Antagonists bind proteins and prevent them from responding to their normal ligands. Binding of tamoxifen by the estrogen receptor stops the receptor from activating transcription of genes that it normally activates.

25. Tamoxifen appears to act by binding the estrogen receptor (I use the terms estrogen receptor and nuclear hormone receptor here as the same thing), with a part of the molecule extending into the region of the protein that normally binds to co-activators. Thus, tamoxifen acts by stopping recruitment by the receptor of co-activators. Tamoxifen is used to treat tumors that are stimulated by the binding of estrogens to the receptor.

26. Altering chromatin structure is an essential function for transcriptional activation in eukaryotes. Co-activator proteins appear to play a role in this process by catalyzing the acetylation of lysine residues in histones. Acetylation of histone lysines neutralizes their positive charge, changing the affinity of histones for DNA and changing the nature of their interaction with DNA, thus allowing more proteins to be able to gain access to the DNA where the acetylation has occurred.

27. Proteins involved in transcriptional control often have bromodomains. These regions of protein recognize and bind to acetylated lysine residues in histones.

28. Altering chromatin structure involves a process called remodeling. Steps in this process include 1) binding of a transcription factor to a promoter sequence; 2) recruitment of co-activators; 3)

Although candle makers protested
The decision could not be contested
Its price is quite steep
If at work they should sleep
There will not be a wick for the rested

acetylation of histone lysines by co-activators; 4) binding of the 'remodeling engine' at the acetylation site; 5) exposing of DNA by the remodeling engine; and 6) binding of RNA polymerase II to the exposed DNA.

29. Attenuation is a regulatory mechanism for several *E. coli* operons involved in amino acid metabolism.

30. The tryptophan operon of *E. coli* (trp operon) controls the expression of genes necessary to make tryptophan. When tryptophan is abundant in *E. coli*, the ribosome moves quickly along the trp operon mRNA, making protein. Control of the operon is set up so that transcription of the entire operon only occurs when tryptophan is limiting in the cells. When tryptophan is abundant, transcription terminations very early. The system is set up such that translation of the operon plays a role in the early transcriptional termination.

31. In eukaryotic cells, the ferritin mRNA has a region of it called an iron response element that can be bound by a protein called IRE-BP (iron response element binding protein).

32. IRE-BP binds the iron response element when iron is absent. If IRE-BP is NOT bound to the iron response element (high iron conditions),

Little Johnny the smartest debater
Pondered which integer was greater
Finding answers demands
That he count on his hands
Such is his digital calculator

The Vision Thing

To the tune of "*Star Spangled Banner*"

Did you know you can see
In the dimmest of light
With your rods and your cones
And their retinaldehyde

Found in rhodopsin it's
Got a bond shaped as cis
But it changes its state
When a photon gets it straight

Then the sign'ling kicks in
Thanks to a transducin
Cuz its GTP ways
Turn on diesterase

So gated ion channels stop
Charges from passing through
Such as sod-i-um plus one
And cal-ci-um plus two

Recorded by David Simmons
Lyrics by Kevin Ahern

ferritin is made because the IRE-BP does not block the ribosome from translating the mRNA.

33. Thus, when iron concentration is high, ferritin is synthesized to hold it. When IRE-BP is bound to the iron response element (low iron conditions), ferritin is not made. So when iron is not available, ferritin is not made. Gene expression of ferritin is therefore a function of translational control.

34. The transferrin receptor has multiple iron response elements at the 3' end of its mRNA. When IRE-BP binds to it, the 3' end is protected and transferrin receptor is made.

35. So, when iron is low, the IRE-BP binds the transferrin receptor mRNA, protecting it, and the transferrin receptor protein is made to bring iron into the cells. When iron is high, the IRE-BP leaves the mRNA's 3' end, leaving it susceptible to degradation. Gene expression of the transferrin receptor is therefore a function of stability of the gene's mRNA.

36. When iron inside the cell is high, ferritin is made to hold onto it and when iron is low inside the cell, transferrin receptor is made to bring more iron in.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Section 3

Appendix - Exams

SECTION III

Exams we have given our students are included in this section. They are hyperlinked below.

1. **Mid-Level Course #1**
   1. **Exam 1** / **Exam Key**
   2. **Exam 2** / **Exam Key**
   3. **Final** / **Exam Key**
2. **Mid-Level Course #2**
   1. **Exam 1** / **Exam Key**
   2. **Exam 2** / **Exam Key**
   3. **Final** / **Exam Key**
3. **Basic Course Exams**
   1. **Exam 1** / **Exam Key**
   2. **Exam 2** / **Exam Key**
   3. **Exam 3** / **Exam Key**
   4. **Final** / **Exam Key**

Exam 1 (BB 450/550)

Section I – Short answer - The questions below can generally be answered in 15 words or less. While you will not be required to use 15 words or less, excessively long answers will be scrutinized closely. Each correctly filled in blank below will be awarded three points, except as noted.

Important items

A. The logarithm of a number greater than one is a positive number.
B. The logarithm of a number less than one is a negative number
C. For any number X (except X=0), ln (X) = -ln(1/X) [or log(x) = -log(1/x)]
D. pH = pKa + log {[Salt]/[Acid]}
E. pH = -log[H^+] , pOH = -log[OH^-], pKa = -Log[Ka], pH + pOH = 14
F. pKa values to assume for amino acids – alpha amine = 10, alpha carboxyl = 2.2, R-group amine = 12, R-group carboxyl = 4.4, R-group sulfhydryl = 8
G. $\Delta G = \Delta G^{\circ\prime}$ + RTLn([Prod]/[React])
H. R (the gas constant) = 8.3x10-3 kJ/Kmol

1. You have an acetate buffer. You add sodium acetate (salt) to it. What happens to the pH?

2. You are examining a 2D gel. What was the name of the first technique that was used to perform this technique?

3. You want to make the smallest polypeptide you can that will have the maximum positive charge without using the same amino acid twice. Give a sequence of such a polypeptide that you would make

4. Name all of the amino acids that cannot exist as zwitterions

5. You have an acetate buffer. You manage to magically destroy half of the sodium acetate in it without converting it into anything else. What happens to the pH of the resulting solution?

6. Tell precisely/completely what one joins together to make a peptide bond. Structures not necessary or desired.

7. Scurvy arises as a result of the structural weakness of what compound in the body?

8. Name the categories described in class that the following amino acids are grouped in

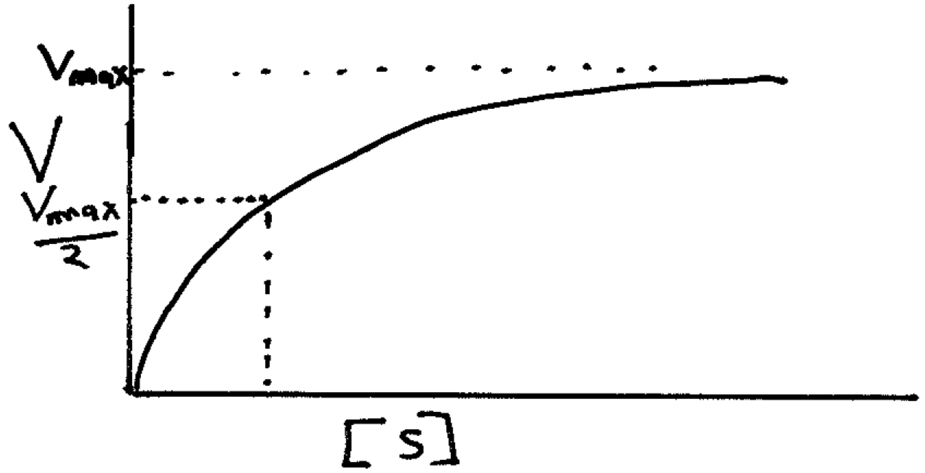
Leucine =

Cysteine =

Asparagine =

9. What chemical named in class can one use to reduce a covalent bond between two cysteine side chains?

10. A scientist studies the kinetic behavior of an enzyme and obtains the following plot. Clearly mark the point on the graph you would use to calculate K_m (2 points) and tell precisely how you would calculate K_m from the value of this point (2 points).



11. Velocity of a car is given in miles per hour. How is the velocity of an enzymatic reaction given? (must be precise)

12. What is the name of the technique described in class uses beads with tiny “tunnels” in them?

13. Name all of the levels/types of protein structure stabilized at least partly by hydrogen bonds

Section 2 – Calculations - For each of the problems in this section, ORGANIZE and LABEL your calculations clearly. No partial credit will be given without clearly labeled calculations.

1. You have 1L of a 0.6M buffer at maximum buffering capacity. When you add 0.1 moles of HCl to the buffer (no volume change), the pH is 9.4. Show how you would calculate the pKa of the buffer.

2. A scientist has the polypeptide shown below at pH = 0 and begins adding NaOH to it.

glycine-arginine-leucine-aspartic acid-methionine-leucine

a. Using the pKa values from the first page of the exam, draw the titration curve (with properly labeled axes) for this molecule up to pH = 14.

b. Indicate the charge on the polypeptide at each relevant place on the graph

c. Show how to calculate the pI of this polypeptide

Section 3 – Explanations –For each question, provide a brief explanation of the phenomenon. Long rambling answers that are not to the point will lose points, even if they contain part of the correct answer.

1. I named at least four molecules/atoms/ions that affect hemoglobin's structure upon binding to it. Name each molecule/atom/ion and describe precisely where it binds on hemoglobin and how it affects oxygen affinity (20 points)

2. Your friend is studying an enzyme in the presence of a non-competitive inhibitor and says that it gives a value of K_{cat} different than the same enzyme without the non-competitive inhibitor. Another friend says that it should have the same K_{cat} as the uninhibited reaction. The head of the lab walks up and says that both statements can be correct, depending on how you calculate K_{cat}. What is the head of the lab thinking? (10 points)

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Exam 2 (BB 450/550)

Section I – Short answer - The questions below can generally be answered in 15 words or less. While you will not be required to use 15 words or less, excessively long answers will be scrutinized closely. Each correctly filled in blank below will be awarded three points, except as noted.

Important items

A. The logarithm of a number greater than one is a positive number.
B. The logarithm of a number less than one is a negative number
C. For any number X (except X=0), ln (X) = -ln(1/X) [or log(x) = -log(1/x)]
D. pH = pKa + log {[Salt]/[Acid]}
E. $pH = -log[H^+]$, $pOH = -log[OH^-]$, pKa = -Log[Ka], pH + pOH = 14
F. pKa values to assume for amino acids – alpha amine = 10, alpha carboxyl = 2.2, R-group amine = 12, R-group carboxyl = 4.4, R-group sulfhydryl = 8
G. $\Delta G = \Delta G°' + RTLn([Prod]/[React])$
H. R (the gas constant) = 8.3x10-3 kJ/Kmol

1. What is the difference between the DNA sequence recognized by a restriction enzyme and the DNA sequence recognized by its corresponding methylase?

2. Carbonic anhydrase is more active at pH 8.5 than it is at pH 7. Precisely what is favored at the higher pH that causes this?

3. I talked about at least three different ions that could act as nucleophiles in enzyme catalysis. Draw three of these (hint – be sure to draw the form of each ion that acts as the nucleophile)

4. Only one of the allosteric effectors of ATCase favors the T-state. Which one?

5. Draw the structure of the first glycolysis intermediate containing two phosphates.

6. Precisely what chemical reaction on what molecule/compound/enzyme does warfarin directly inhibit (four points)?

7. Define the term ‘anomers’

8. Draw the Fischer structure of and name the only pentose you are responsible for knowing the structure of (no partial credit)

9. What is the name of the only enzyme of glycolysis that catalyzes a redox reaction?

10. In the catalytic action of serine proteases, what must happen to move the catalytic triad closer together to create the alkoxide ion and start the catalytic process?

11. Where in the cell are O-linked glycoproteins made?

12. What are the name(s) of the second/third messenger(s) for the pathway involving phospholipase C?

13. Name a compound described in class that is an inhibitor of BCR-ABL

Section 2 – Calculations - For each of the problems in this section, ORGANIZE and LABEL your calculations clearly. No partial credit will be given without clearly labeled calculations. (15 points each)

1. A reaction is at equilibrium at a temperature of 300K with a [Reactant] = .4M and [Product] = 1.0M. Using as much of this information as possible, write an equation to calculate the ΔG when there are equal amounts of the two (15 points)

2. The reaction below meets the needs of the body's muscles remarkably well. Explain how the G for this reaction varies according to whether one is exercising or resting and how that provides the muscles with what they need to keep the body functioning. (15 points)

Creatine + ATP <=> Creatine phosphate + ADP

($\Delta G°'$ = + 12 kJ/mol)

Section 3 – Explanations –For each question, provide a brief explanation of the phenomenon. Long rambling answers that are not to the point will lose points, even if they contain part of the correct answer.

1. You have four friends. Two are born without alpha-1-antitrypsin and two are born with a normal alpha-1-antitrypsin. Their circumstances are below:

A. No alpha-1-antitrypsin - smokes
B. No alpha-1-antitrypsin - doesn't smoke
C. Regular alpha-1-antitrypsin - smokes
D. Regular alpha-1-antitrypsin - doesn't smoke

Predict the relative tendency to have emphysema you would expect for each of the friends in the comparison below based on class discussion. Justify your answer at the end of the problem. (15 points)

a. Compare A to B (is emphysema in A > B, A<B, or A=B)

b. Compare C to D (is emphysema in C > D, C<D, or C=D

c. Compare B to D (is emphysema in B > D, B<D, or B=D)

2. You are walking home one night when out of the bushes jumps your biochemistry professor singing loudly and very off key. This scares you terribly, so you start secreting the same hormone you would if a grizzly bear were chasing you. Name the hormone and show/describe the entire signaling pathway described in class that it activates. (15 points)

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Final Exam (BB 450/550)

Section I – Short answer - The questions below can generally be answered in 15 words or less. While you will not be required to use 15 words or less, excessively long answers will be scrutinized closely. Each correctly filled in blank below will be awarded three points, except as noted.

Important items

A. The logarithm of a number greater than one is a positive number.
B. The logarithm of a number less than one is a negative number
C. For any number X (except X=0), ln (X) = -ln(1/X) [or log(x) = -log(1/x)]
D. pH = pKa + log {[Salt]/[Acid]}
E. pH = -log[H^+] , pOH = -log[OH^-], pKa = -Log[Ka], pH + pOH = 14
F. pKa values to assume for amino acids – alpha amine = 10, alpha carboxyl = 2.2, R-group amine = 12, R-group carboxyl = 4.4, R-group sulfhydryl = 8
G. $\Delta G = \Delta G^{\circ\prime}$ + RTLn([Prod]/[React])
H. R (the gas constant) = 8.3x10-3 kJ/Kmol

1. One method of separating proteins described in class combines two different separation techniques. Name the overall method and the names of the two separation techniques

2. Name all of the amino acids described in class that can be phosphorylated

3. Describe or illustrate (precisely) below the reaction that mercaptoethanol catalyzes

4. Velocity of a car is given in miles per hour. How is the velocity of an enzymatic reaction given? (must be precise)

5. Name three forces stabilizing quaternary interactions

6. A deficiency of what dietary molecule causes scurvy?

7. Name the only molecule described in class that competes with oxygen for the binding site on hemoglobin

8. To what atom does oxygen bind in hemoglobin?

9. Precisely where on ATCase does aspartate bind?

10. What is the name of the enzyme in glycolysis that produces an intermediate that affects hemoglobin's affinity for oxygen?

11. Name the only enzyme in glycolysis that is regulated both allosterically and by covalent modification

12. Draw the Haworth structure for the only hexose given in class for which you must know the furanose form

13. What is the name of the enzyme that catalyzes the breakdown of blood clots?

14. In the catalytic action of serine proteases, what must happen to move the catalytic triad closer together to create the alkoxide ion and start the catalytic process?

15. Gleevec inhibits Bcr-Abl. What enzymatic reaction does this protein catalyze?

16. What bond is broken by the nucleophilic attack in the fast step of the catalytic mechanism of serine proteases?

17. Name three first messengers described in class

18. A friend tells you that a new buffer has been discovered in which there is more salt than acid when the pH is the same as the pKa. Using math, explain why this is true or false. (Your answer must also say “TRUE” or “FALSE”)

19. Kevin Ahern’s hypothesis of why Americans are becoming more and more obese says it is due to the bypassing of an important enzymatic reaction. What is the name of this enzyme?

20. What molecule is synthesized in animals in order to make NAD when oxygen levels are low?

21. In utilization of galactose, it is first phosphorylated and added to a molecule that results in the release of glucose-1-phosphate. To what molecule is the phosphorylated galactose attached?

22. Name a transcription factor made/activated in cells when oxygen is lacking.

23. Name a molecule that activates an enzyme in feed-forward activation.

24. Name the only enzyme I described in class that is used both in the breakdown of glycogen pathway and the synthesis of glycogen pathway

25. What does it take to convert glycogen phosphorylase a from the T state to the R state?

Section 2 – Calculations - For each of the problems in this section, ORGANIZE and LABEL your calculations clearly. No partial credit will be given without clearly labeled calculations. (12 points each)

1. You have 1L of a 0.6M buffer with twice as much salt as acid. After you add .1 moles of HCl (no volume change) and raise the temperature of the solution by 10 degrees, the pH is 8.32. Write an equation to calculate the pH before the HCl was added.

2. A reaction starts with 5 times as much reactant as product, but at equilibrium, it only has 3 times as much reactant as product.

    a. Write an equation to show how to calculate ΔG°’ (3 points)

    b. Write an equation to show how to calculate ΔG at the very beginning (3 points)

    c. Show how you would determine the sign of the ΔG°’ (3 points)

d. What was the sign of ΔG at the start of the reaction? Why? (3 points)

3. An analysis of an enzymatic reaction is performed in the presence and absence of an inhibitor. The inhibitor appears to reduce the V_{max} of the enzyme.

a. Draw a single Lineweaver Burk plot showing the uninhibited and the inhibited reaction. Clearly label all relevant features on the graph. (5 points)

b. Draw a single V vs. [S] plot for the showing the uninhibited and the inhibited reaction. Clearly label all relevant features on the graph. (5 points)

c. What type of inhibition is occurring? (2 points)

Section 3 – Explanations –For each question, provide a brief explanation of the phenomenon. Long rambling answers that are not to the point will lose points, even if they contain part of the correct answer.

1. Regulation of glycolysis and gluconeogenesis is complex. It involves allosteric interactions, covalent modifications, and control of enzymes that make/break down moleules that affect the pathway.

a. Name the enzymes regulated in each pathway (7 points)

b. Name one allosteric activator and one allosteric inhibitor of each enzyme that is reciprocally regulated (4 points)

c. Name the most important allosteric regulator of the two pathways and show precisely how its synthesis and breakdown is regulated. (6 points)

2. Glycogen phosphorylase is regulated allosterically and by an enzyme it carries around with it. Draw a scheme illustrating all of the ways in which glycogen phosphorylase is regulated and also a scheme showing how the enzyme it carries can affect the enzyme. (10 points total)

3. We have talked this term about three broad ways in which enzyme activity can be regulated. Name each way. Name one enzyme regulated each way. Give detail as appropriate for each means of regulation. If external molecules or modifications are relevant, your answer must include them and a description must include the effect the molecules or modifications have on each enzyme. (12 points)

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Exam 1 (BB 451/551)

Section I: (20 points total) The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive four points if you circled 'b','c', and 'd'. You would receive one point if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings.

Practice question #A: Oregon State University

A. is a Peruvian factory
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world.

1. With respect to the citric acid cycle,
   A. succinate dehydrogenase requires FAD for catalysis
   B. it includes two decarboxylations not found in the glyoxylate cycle
   C. NADH is not used
   D. it uses oxygen in a one of its enzymatic reactions

2. With respect to respiratory control,
   A. cyanide will stop oxidative phosphorylation first
   B. exercising will favor production of NAD^+
   C. resting will favor decrease in ADP
   D. 2,4 DNP (miracle diet drug) will increase NADH and temperature

3. With respect to membrane transport,
   A. lactose permease is an anti-port
   B. it requires ATP
   C. it requires diffusion
   D. it cannot be electroneutral if sodium is involved

4. With respect to electron transport,
   A. electrons get pumped into the intermembrane space
   B. oxygen is the terminal proton acceptor
   C. the Q cycle occurs in Complex III
   D. 2,4 DNP will increase oxygen consumption

5. In tightly coupled mitochondria,
   A. increasing oxygen will increase the proton gradient
   B. lack of NAD will ultimately reduce electron flow
   C. uncoupling protein (UCP) will reduce ATP production
   D. the citric acid cycle will run fastest when electron transport runs fastest

Section II: (50 points total) Each sentence below in this section is missing a word or phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded two points.

1. Name the five carbon intermediate in the citric acid cycle

____

2. Which enzyme of the citric acid cycle catalyzes a reaction that pulls a reaction preceding it? ______________________

3. Which enzyme of the citric acid cycle requires lipoamide?

____

4. Which enzyme of the citric acid cycle catalyzes the only oxidation that doesn't produce NADH? ______________________

5. What citric acid cycle intermediate can be readily made from glutamic acid in an anaplerotic reaction?

____

6. How many oxaloacetates are produced per turn of the citric acid cycle? ______________________

7. Name a poison described in class that reacts with a citric acid cycle coenzyme ______________________

8. Which class of membrane-related proteins would be the most likely to have hydrophobic amino acids on its exterior?

____

9. If one membrane protein pump were missing from the cell, neural transmission as described in class would not be possible. What pump is that? ______________________

10. What is the basis for exclusion of potassium from a sodium channel? ______________________

11. What is the basis for exclusion of sodium from a potassium channel? ______________________

12. Active transport always has at least one mechanistic difference from passive transport. It is the reason that active transport requires energy. What is that difference?

____

13. What is the driving force for passive transport?

______________________.

14. What is the definition of a symport?

______________________.

15. You remove digitoxigenin from a heart cell that had been treated with it. What happens to the concentration of sodium outside of the cell? ______________________.

16. In the Q cycle, what takes electrons away from Complex III?

______________________.

17. Complex V has a rotating complex that make ATP. It has three different configurations. What is the form of the configuration where ATP is released? ______________________.

18. You treat a mitochondrion 2,4 DNP and cyanide. What happens to oxygen consumption? ______________________.

19. A mitochondrion is treated with 2,4 dinitrophenol (2,4 DNP). What happens to its oxygen consumption?
______________________.

20. The mitochondria in a cell are tightly coupled. The citric acid cycle is running furiously. What normal explanation (not exotic or unusual) makes the most sense about what this cell is doing to cause this? ______________________.

21. If one wanted to make a photosynthetic fish, as described in class, what protein would you need to do it?
______________________.

22. Describe the numbering system for an omega fatty acid. Which carbon is number one? ______________________.

23. What is the name of the category of ATP-using transport proteins that involve a phosphoaspartate?
______________________.

24. What is the charge on a copper atom in Complex IV after it has accepted an electron? ______________________.

25. If you wanted to raise the T_m of a membrane, how would you alter the chemical composition of its fatty acids?
______________________.

Section III: (30 points total) Matching.

____ 1. Na/K ATPase
____ 2. Isocitrate
____ 3. Brown fat
____ 4. Acetaldehyde
____ 5. Decarboxylation
____ 6. Sphingomyelin
____ 7. Malate synthase
____ 8. Antimycin A
____ 9. Oxygen
____ 10. ADP
____ 11. H_2O_2
____ 12. Coenzyme Q
____ 13. Synaptic vesicles
____ 14. Malate
____ 15. Thiamine pyrophosphate (TPP)

A. Can stop oxygen consumption indirectly
B. Limiting for people not exercising
C. Produced by superoxide dismutase
D. Glyoxylate cycle requirement
E. Carries electrons in pairs
F. Five carbon intermediate
G. Phosphorylated membrane component
H. Neurotransmitter described in class
I. Neurotransmitter holder
J. Complex IV inhibitor
K. Superoxide dismutase product
L. Process that does not occur in glyoxylate cycle
M. Six carbon intermediate
N. Symport
O. Blocked by tetrodotoxin
P. Produced by citrate synthase
Q. Gets oxidized after being shuttled
R. Ethanol precursor
S. Produced by oxidative phoshorylation
T. Electro-genic
U. Can become uncoupled
V. Carries a two carbon group

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Exam 2 (BB 451/551)

Section I: (20 points total) The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive four points if you circled 'b','c', and 'd'. You would receive one point if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings.

Practice question #A: Oregon State University

A. is a Peruvian factory
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world

1. With respect to cholesterol metabolism,
   A. it uses palmitoyl-CoA and serine
   B. it includes an intermediate of squalene
   C. ATP is not used
   D. it is a precursor to making vitamin D, bile acids, and steroid hormones

2. With respect to fatty acid metabolism,
   A. it starts with oxidation of medium chain fatty acids in peroxisomes
   B. oxidation of saturated fatty acids involves cis intermediates
   C. enoyl-CoA isomerase is not needed for oxidation of saturated fatty acids
   D. ketone bodies use the last enzyme of fatty acid synthesis

3. With respect to prostaglandins,
   A. ibuprofen is a COX-1 inhibitor
   B. steroids inhibit COX-2 enzymes
   C. they can be produced from thromboxanes or leukotrienes
   D. PLA-2 inhibits the action of aspirin

4. With respect to nucleotide metabolism,
   A. deoxyribonucleotides are made from ribonucleoside diphosphates
   B. PRPP amidotransferase balances relative amounts of purines
   C. NDP Kinase (NDPK) converts ADP to dADP
   D. dUTPase converts dUTP to dTTP

5. With respect to DNA replication,
   A. the beta clamp is responsible for making DNA Polymerase III more processive
   B. helicase forms double helices at the rate of 6000 rpm
   C. Okazaki fragments are only made in the 5' to 3' direction
   D. lagging strand replication occurs in the 3' to 5' direction

Section II: (50 points total) Each sentence below in this section is missing a word or phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded two points.

1. What is the name of the transcription factor that helps control synthesis of HMG-CoA reductase? ______________________

2. I named four general mechanisms for regulating HMG-CoA reductase. Name three of them (1 point each)
______________________, ______________________,
____

3. What group of molecules has their synthesis stopped by aromatase inhibitors? ______________________

4. I mentioned three factors to consider for controlling the level of cholesterol in the body. Name them (1 point each)
______________________, ______________________,
____

5. What is the name of the cellular structure missing in familial hypercholesterolemia? ______________________

6. What is the name of the last product of beta oxidation of a fatty acid with an odd number of carbons?
______________________ (name must be precise)

7. Name the only regulated enzyme in either fat or fatty acid breakdown ______________________

8. Draw the structure that the enzyme thiolase would cut
____

9. Describe accurately in words what enoyl-CoA isomerase catalyzes

10. Name the enzyme that catalyzes the first reaction in the pathway leading to ketone bodies ______________________

11. Name the molecule produced by catalysis by the only regulated enzyme of fatty acid biosynthesis
____

12. Name the molecule that shuttles the precursor of fatty acid synthesis to the cytoplasm ______________________

13. How does carbamoyl phosphate synthetase protect its intermediate product from water? ______________________.

14. What is the name of the enzyme that balances purines and pyrimidines in de novo ribonucleotide synthesis?
______________________.

15. What molecule is the energy source for synthesis of AMP?
______________________.

16. What is the name of the molecule that is the branch point in *de novo* purine ribonucleotide synthesis?
______________________.

17. Write out the reaction catalyzed by adenylate kinase
______________________.

18. What atom does RNR (ribonucleotide reductase) catalyze the removal of? ______________________.

19. What is the name of the electron source for fatty acid biosynthesis? ______________________.

20. What is the name of the first electron acceptor in fatty acid oxidation? ______________________.

21. Name the form of DNA discovered by Rosalind Franklin ______________________.

22. Name the enzyme necessary for joining the DNAs of Okazaki fragments ______________________

22. What is the name of the first protein to bind to the E. coli DNA replication origin? ______________________.

23. Precisely what does telomerase use as a template to make telomeres? ______________________.

24. What enzymatic activity is present in DNA polymerase I and III, but lacking in reverse trancriptase of HIV? ______________________.

25. Give the ratio that determines whether or not a DNA is relaxed or not (note – I am looking for the units, not a number) ______________________.

Section III: (30 points total) This section of the exam is on the next page.

Matching.

Each term on the left has a phrase or term on the right which best describes or matches it. Place the letter of the term/phrase on the right in the blank before the term on the left that it best matches. Only one letter is appropriate in the blank. Note that there are more terms on the right than there are blanks, so not every term on the right has a best match. Terms on the right may be used once, more than once, or not at all. If we cannot read your writing or if you put two letters in any blank on the left, your answer will be counted wrong automatically. Each correctly matched pair is worth two points.

____ 1. dnaB
____ 2. Guanosine
____ 3. Gyrase
____ 4. Arachidonic acid
____ 5. LDL
____ 6. Serine
____ 7. CDP
____ 8. Cholesterol
____ 9. dATP
____ 10. S-Adenosyl Methionine (SAM)
____ 11. HMG-CoA
____ 12. Lipase
____ 13. Ganglioside
____ 14. ACP
____ 15. Glycerol

A. Necessary for fat digestion
B. Lipoprotein complex
C. Palmitate precursor
D. Requires vitamin B_{12}
E. Replaces CoA of malonyl-CoA
F. Feedback inhibitor
G. ATCase inhibitor
H. Lacking in Lesch-Nyhan syndrome
I. Leukotriene precursor
J. Sphingolipid
K. Prostaglandin product
L. Topoisomerase
M. Nucleoside
N. Energy source in activated intermediates
O. Branch point molecule for two pathways
P. Necessary for sphingolipid synthesis
Q. Glycerophospholipid
R. Methyl donor
S. INSIG target
T. Can be made into glucose
U. Helicase
V. Allosteric regulator of ribonucleotide reductase

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Final Exam (BB 451/551)

Section I: (40 points total) The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive four points if you circled 'b','c', and 'd'. You would receive one point if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings.

Practice question # A: Oregon State University

A. is a Peruvian factory
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world.

1. With respect to the citric acid cycle,
   A. GDP is not required
   B. lipoic acid is not used
   C. oxygen is not a substrate in the reactions of the cycle
   D. oxidative decarboxylation does not occur

2. With respect to movement of compounds across biological membranes,
   A. active transport requires ATP
   B. water cannot readily cross them
   C. a proton gradient is required
   D. protons generally require a protein to move across them

3. In tightly coupled mitochondria,
   A. oxidative phosphorylation will speed up to make up for an oxygen deficit
   B. low ADP levels will have the same overall effect as low oxygen levels
   C. increasing intermembrane space pH will reduce ATP production
   D. cyanide will reduce both ATP production and the proton gradient

4. With respect to the Q cycle,
   A. it explains how electrons come in pairs, but are converted to moving singly
   B. it involves coenzyme Q in three different forms
   C. Cytochrome C carries electrons to complex III
   D. each QH_2 sends electrons to two different things

5. With respect to fatty acid oxidation,
   A. it occurs in the cytoplasm and the peroxisomes
   B. it produces $FADH_2$ and NADH
   C. it only occurs on fatty acids shorter than 16 carbons
   D. it occurs when the fatty acid is bound to ACP

6. With respect to fatty acid synthesis,
   A. it is allosterically activated by palmitate/palmitoyl-CoA
   B. it occurs in the cytoplasm and endoplasmic reticulum
   C. it uses malonyl-ACP
   D. it requires a decarboxylation

7. With respect to prostaglandins,
   A. aspirin is a COX inhibitor
   B. inhibiting phospholipase A_2 will stop release of fatty acids from membranes
   C. they are precursors of cholesterol
   D. they are pain relievers

8. With respect to nucleotide metabolism,
   A. it uses ribonucleotide reductase to convert all dNDPs to dNTPs
   B. it uses CTP to make UTP
   C. it uses dTMP to make dUMP
   D. it uses DHFR to make dNDPs from NDPs

9. With respect to transcription,

A. it occurs at a structure called a replication fork
B. the template strand is complementary to the one being made
C. it makes RNA in the 3′ to 5′ direction
D. it requires a ribosome

10. With respect to gene expression,

A. attenuation involves early transcriptional termination
B. the lac repressor binds to DNA when it binds allo-lactose
C. CAP binds to DNA when it binds to cAMP
D. IRE-BP binds to DNA when it does not bind iron

Section II: (80 points total) Each sentence below in this section is missing a word or phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded two points.

1. What coenzyme named in class does arsenate react with?

____

2. Which enzyme of the citric acid cycle catalyzes a reaction that produces $FADH_2$? ________________________

3. What is the definition of an antiport? ________________________.

4. Which enzyme of the citric acid cycle catalyzes a reaction that pulls the one preceding it? ________________________

5. What amino acid is alpha-keto-glutarate readily converted to in an anaplerotic reaction? ________________________

6. Name one enzyme unique to the glyoxylate cycle

____

7. You take a heart cell that was treated with digitoxigenin and remove the digitoxigenin. What happens to the concentration of calcium outside of the cell? ________________________.

8. Name a specific sphingolipid named in class that contains a phosphate

____

9. Name the class of lipid described in class that contains complex carbohydrates ________________________

10. A gradient of which ion is used as an energy source to remove calcium ions from heart cells? ________________________.

11. What is the criterion we use to determine if a transport mechanism is active versus passive? ________________________.

12. In what class of membrane transporters does phosphoaspartate appear?

________________________.

13. A cell (and its mitochondria) are treated with 2,4 dinitrophenol (2,4 DNP). What happens to the citric acid cycle in this cell?

________________________.

14. Name one of the two isoprenes described in class

_______________________.

15. Complex V has a rotating complex that makes ATP. It has three different configurations. What is the form of the configuration where ATP is released? _______________________.

16. Describe precisely the catalytic activity of DNA Polymerase I that removes RNA primers _______________________.

17. Precisely what does telomerase copy to make a telomere?

_______________________.

18. How does one completely inhibit PRPP amidotransferase?

_______________________.

19. What is the name of the molecules that donate single carbons in nucleotide metabolism (hint – I'm not looking for carbon dioxide or bicarbonate) ?

_______________________.

20. What molecule does aspirin prevent from being acted on by COX?

_______________________.

21. In fatty acid synthesis, describe the chemical step after deyhdration (all I need is a name describing the chemical reaction) _______________________.

22. Name the enzyme that catalyzes conversion of fatty acids with a *cis* double bond between carbons 3/4 to a *trans* double bond between carbons 2/3

_______________________.

23. What causes gout? _______________________.

24. Where does one find acyl-CoA dehydrogenases that act on fatty acids of 20 carbons? _______________________.

25. Name a covalent modification of an enzyme in fatty acid metabolism and the effect of that covalent modification

Enzyme modified = _______________________.

Modification = _______________________.

Effect of modification _______________________.

26. Name the enzyme used in DNA footprinting _______________________.

27. Name the specific catalytic entity that forms phosphodiester bonds between ribonucleotides _______________________.

28. Name the specific catalytic entity that forms peptide bonds between amino acids in bacteria _______________________.

29. Define the term "template strand" _______________________.

30. Name the protein described in class that "spins" at 6000 rpm

______________________.

31. In attenuation of the trp operon, what is the function of the structure in the mRNA that only forms when there is abundant tryptophan in the cell?

______________________.

32. What does tamoxifen prevent the estrogen receptor from interacting with? ______________________.

33. In elongation, which direction is the ribosome moving relative to the mRNA? ______________________.

34. Name the 7TM of vision that is bound to Vitamin A

______________________.

35. Name the G protein involved in the sense of smell

______________________.

36. Describe the unusual bond in a lariat structure

______________________.

37. Describe what rho does and how it does it ______________________.

38. Precisely where is the wobble base of the anticodon found ?

______________________.

Section III: (30 points total) Matching. Each term on the left has a phrase or term on the right which best describes or matches it. Place the letter of the term/phrase on the right in the blank before the term on the left that it best matches. Only one letter is appropriate in the blank. Note that there are more terms on the right than there are blanks, so not every term on the right has a best match. Terms on the right may be used once, more than once, or not at all. If we cannot read your writing or if you put two letters in any blank on the left, your answer will be counted wrong automatically. Each correctly matched pair is worth two points.

____ 1. TPP
____ 2. Intron
____ 3. Transducin
____ 4. Gyrase
____ 5. dATP
____ 6. Inducer
____ 7. Thiolase
____ 8. Statin
____ 9. Active transporter
____ 10. Tamoxifen
____ 11. Trans fat
____ 12. Okazaki fragments
____ 13. Carnitine

____ 14. Anti-codon
____ 15. Ribozyme

A. Peptide bond catalyst(s)
B. Purine breakdown product(s)
C. Binds retinal
D. Hydrogenation byproduct(s)
E. tRNA component(s)
F. Sphingolipid(s)
G. Lagging strand synthesis product(s)
H. IPTG
I. G protein(s)
J. Ketone body enzyme(s)
K. Prostaglandin enzyme(s)
L. Na/K ATPase
M. Pyruvate dehydrogenase complex component(s)
N. pre-mRNA component(s)
O. Reverse transcriptase(s)
P. Topoisomerase(s)
Q. Helicase(s)
R. Translation chain terminator(s)
S. Copper
T. Mitochondrial membrane crosser(s)
U. Ribonucleotide reductase inhibitor(s)
V. HMG-CoA reductase inhibitor
W. Fatty acid synthase end product(s)
X. Estrogen receptor binder(s)

Exam 1 (BB 350)

Section I: The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive eight points if you circled 'b','c', and 'd'. You would receive two points if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings.

Practice question #A: Oregon State University
- A. is a factory in Portland
- B. is located in Corvallis, Oregon
- C. has a mascot named Benny Beaver
- D. has students from all over the world.

1. With respect to hydrogen bonds,
    - A. they are weaker than covalent bonds
    - B. they are weaker than disulfide bonds
    - C. they are what make peptide bonds
    - D. water does not have to be involved

2. With respect to amino acids,
    - A. glycine's R group is a hydrogen
    - B. lysine's R group is more hydrophobic than leucine's
    - C. methionine is the only one with a sulfur in the R group
    - D. every one of them can exist as a zwitterion

3. With respect to enzymes,
    - A. Km is a velocity
    - B. Vmax/2 is the same as Km
    - C. Kcat varies with substrate concentration
    - D. Kcat = Km /[enzyme]

4. With respect to information in the syllabus,
    - A. there is no fixed grading scale
    - B. grades are curved
    - C. the grading scale is 90/80/70/60

Section II: 1. Each sentence below in this section is missing a word or short phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded three points. You MUST be precise in your definitions.

1. Describe the difference between a strong acid and a weak acid. _______________

2. Name an amino acid that makes disulfide bonds _______________

3. How does a fibrous protein differ from a globular protein? _______________

4. A solution has a pH of 6. Write an equation for its hydroxide ion concentration _______________

5. What is the name of the group in myoglobin that contains the iron? _______________

6. Define allosterism ________________

7. You have a buffer solution with more salt than acid and the pH is 6. What can you say about the pKa? ________________

8. Explain from an energetic perspective how enzyme catalysis works (one sentence should suffice).

9. What units does K_M have? ________________

10. Name three separation techniques described in class

11. What name do we give to the molecule(s) that an enzyme catalyzes a reaction on? ________________

Section III – Problems/Long Answer

1. Explain at the molecular level why smokers breathe more heavily when exercising than non-smokers. (16 points)

2. An exotic amino acid is discovered that has two amine groups and three carboxyl groups. The pKa values of the groups are below

Amino #1 = 8.8
Amino #2 = 12.1
Carboxyl #1 = 1.1
Carboxyl #2 = 4.2
Carboxyl #3 = 6.6

a. Draw and label CLEARLY a titration curve with all relevant values for this amino acid (12 points)

b. Calculate the approximate charge this amino acid would have at a pH of 5.5 (4 points)

3. For the following problem, ORGANIZE and LABEL your calculations clearly. No partial credit will be given without clearly labeled calculations. You do NOT have to enter the numbers into a calculator. You simply need to show the last step before punching it in.

You have a 500 ml of a .6M buffer of ahernium (pKa = 9.11) in which the concentration of acid is twice that of the salt.

a. Write an equation to calculate the pH of this buffer (4 points)

b. If you wish to get the buffer to maximum buffering capacity, describe whether you would use HCl or NaOH and how many moles you would use. You will need to clearly show and label your calculations to explain your answer. (16 points)

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Exam 2 (BB 350)

Section I: The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive eight points if you circled 'b','c', and 'd'. You would receive two points if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings.

Practice question #A: Oregon State University

A. is a factory in Portland
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world.

1. With respect to fatty acids,
   A. more unsauration lowers their melting temperatures
   B. greater length raises their melting temperatures
   C. oils have more unsaturated fatty acids than fats
   D. fish membranes have more unsaturated fatty acids than humans

2. With respect to nucleic acids,
   A. adenine does not contain a sugar
   B. nucleosides do not contain a sugar
   C. nucleotides do not contain a phosphate
   D. RNA contains ribose, but not deoxyribose

3. With respect to membranes,
   A. anchored membrane proteins project through both sides of the lipid bilayer
   B. liposomes are not natural cellular membranes
   C. carbon dioxide cannot cross the lipid bilayer
   D. cholesterol raises the transition temperature of a membrane

4. With respect to control of enzyme activity,
   A. covalent modification always activates enzymes
   B. allosterism always inactivates enzymes
   C. feedback inhibition works allosterically

Section II: 1. Each sentence below in this section is missing a word or short phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded three points. You MUST be precise in your definitions.

1. What molecule found in the membranes of cells widens the cells' transition temperatures (T_m values)? _______________

2. Define allosterism (no points for "being close")

____

3. ATCase has two different kinds of subunits. What category of subunit does aspartate bind to? _______________

4. Carbohydrates are only found attached to the outside of cell membranes. What is their function, as described in class?

____

5. What is the driving force for the movement of molecules in facilitated diffusion? _______________

6. What three carbon molecule forms the backbone of glycerophospholipids, but not sphingolipids? _______________

7. What is required for a membrane transport system to be described as ‘active transport’? _______________

8. Name an inhibitor of prostaglandin synthesis

____

9. What chemical change happens to vitamin A that results in nerve signaling? _______________

10. What is the difference between a deoxyribonucleotide and a deoxyribonucleoside (be precise in your answer)?

11. In attenuation of the trp operon, when there is sufficient tryptophan, what process is terminated? _______________

Section III – Problems/Long Answer

1. *E. coli* DNA polymerase has three essential activities for DNA replication. Name and explain the enzymatic activity that each one catalyzes. (16 points)

2. Draw out the central dogma and identify the exception to it with respect to retroviruses, such as HIV

3. I described in class how *E. coli* can switch from making one class of genes to making another class of genes, due to changes of circumstances. Describe precisely what is involved in making this switch (hint – this has nothing to do with lactose).

4. The lac operon has controls that allow it to adapt to the cell’s needs for energy. Explain all of the controls listed in class and how they allow for this adaptation.

5. List and describe three modifications to eukaryotic mRNAs that do not happen to prokaryotic mRNAs.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Exam 3 (BB 350)

Section I: The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive eight points if you circled 'b','c', and 'd'. You would receive two points if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings.

Practice question #A: Oregon State University
A. is a factory in Portland
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world.

1. With respect to tools for biotechnology,
A. a plasmid is a circular DNA that replicates in human cells
B. a polylinker is a short stretch of DNA in a plasmid with multiple restriction sites
C. enzymes used to cut DNAs at specific places are called methylases
D. a plasmid for making protein in a cell would have to have a promoter

2. With respect to sugar structures,
A. a hexose cannot be a furanose
B. the Fischer straight chain structure of glucose has no anomeric carbon
C. a reducing sugar is easily reduced
D. a glycoside always has a bond involving the anomeric carbon

3. With respect to translation,
A. the 23s rRNA catalyzes the formation of peptide bonds
B. initiation does not involve any peptide bond formation
C. EF-Tu is involved in translocation
D. ATP is required for translocation

4. With respect to polysaccharides,
A. cellulose is a polymer of glucose
B. chitin is a polymer of fructose
C. bacterial cell walls contain peptidoglycan structures with D-amino acids

Section II: 1. Each sentence below in this section is missing a word or short phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded three points, except as noted. You MUST be precise in your definitions.

1. What is the name of the bacterial protein factor responsible for translocation during translation? _______________

2. Precisely where is the wobble base of the anti-codon?
____

3. Describe the physical appearance of a messenger RNA during eukaryotic translation _______________

4. Give the sequence (5' to 3') of the three stop codons (1 point each)

_______________, _______________, and _______________

5. What is the precise name of the first amino acid put into bacterial proteins? (must be exact) _______________

6. What is the function of a chaperonin? _______________

7. What is the precise name we give to a gene that after it is mutated can cause a cancer? _______________

8. Ras causes uncontrolled growth after it is mutated. What does the mutation in ras cause it to be unable to catalyze?

____

9. Name one electron carrier described in class in its oxidized form _______________

10. Describe how one makes a sugar alcohol _______________

11. Describe the structural difference between amylose and amylopectin

12. What animal polysaccharide is similar to amylopectin?

____

13. Draw the structure of L-glucose in the Fischer form

14. Draw the structure of alpha-D-ribose as a furanose

15. What important molecule is an intermediate in the reaction catalyzed by phosphoglycerate mutase? _______________

16. Name the animal product of fermentation _______________

17. What pathway described in class is an important source of NADPH? _______________

Section III – Problems/Long Answer

1. The initiation of prokaryotic translation is a very intricate process in which multiple factors must come together to prepare for the elongation phase. Describe all of the factors described in class, the order in which they come together, and the final structure at the end of initiation. If you list steps in the elongation phase, you will lose points.

2. A reaction goes as follows

Student <=> Instructor

The reaction goes forward when there are equal amounts of Student and Instructor. Similarly, the reaction goes forward when there is ten times as much Student as there is Instructor. Is it possible for the reaction to go forward when there is ten times as much Instructor as Student? If yes, you must state why so. If no, your answer must similarly answer why so. If it is impossible to answer, your answer must also state why so. Any answer you give absolutely be based mathematically in energy. Answers without a mathematical justification will receive no credit.

3. The Cori cycle helps the body to adapt to important situations. Describe the situation(s) it helps to adapt to. In addition, show the relevant places in the body where the reactions occur and show what the products of each relevant organ of the body is. A diagram will be helpful.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Final Exam (BB 350)

Section I: The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive four points if you circled ‘b’,’c’, and ‘d’. You would receive one point if you circled ‘a’ and ‘b’. You would receive no points if you circled only ‘a’. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings.

Practice question #A: Oregon State University
A. is a factory in Portland
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world.

1. Hydrogen bonds
A. are weaker than covalent bonds
C. occur as a result of ionization
D. are the bonds formed between cysteines
E. stabilize secondary, tertiary, and quaternary structures

2. With respect to amino acids,
A. glycine is smaller than proline
B. all of them have a pI
C. biological ones are almost always in the D configuration
D. they can be acted upon by transaminases

3. With respect to membrane lipids,
A. sphingolipids contain glycerol
B. glycerophospholipids do not contain fatty acids
C. sphingolipids are common in brain tissue
D. cholesterol is common in brain tissue

4. With respect to mRNAs,
A. they carry the anti-codon
B. there are 64 of them in theory
C. they contain stop codons
D. they have caps in prokaryotes

6. With respect to viruses,
A. HIV is a retrovirus
B. they sometimes carry oncogenesthey can use reverse
C. transcriptase to make RNA from
RNA
D. they can infect prokaryotes and eukaryotes

7. With respect to lipid metabolism
A. fats are more reduced than carbohydrates
B. fatty acid synthesis occurs in the cytoplasm
C. ACP carries fatty acids across the inner mitochondrial membrane
D. fat breakdown is stimulated by insulin

8. With respect to photosynthesis,
A. protons are pumped outside the chloroplast
B. oxygen is the terminal electron acceptor
C. ATP is produced by substrate level phosphorylation
D. oxygen is produced in the dark cycle

Section II: 1. Each sentence below in this section is missing a word or short phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded three points. You MUST be precise in your definitions.

1. What does one have to remove in order to convert a weak acid into a salt? ________________

2. Which amino acid in the catalytic triad of chymotrypsin gains a proton transiently during the catalytic cycle? ________________

3. Explain why myoglobin does not exhibit cooperativity

____

4. Define cooperativity ________________

5. How does the pKa of a very weak acid compare to the pKa of a weak acid ?

________________ (answer must be clear)

6. Explain precisely the piece of information that one would always know if one had the K_m for an enzyme

____

7. Name the strongest force stabilizing tertiary structure

____

8. Which strand in DNA replication contains the Okazaki fragments? ________________

9. What is the name of the sequence a few bases before the *E. coli* (bacterial) translational start site? ________________

10. What enzyme present in the developing embryo 'builds' the ends of the linear eukaryotic chromosomes? ________________

11. What trait commonly associated with a plasmid allows a researcher to easily identify which cells get the desired DNA during transformation? ________________

12. What is the name of the enzyme that is essential for removing primers in *E. coli* DNA replication? ________________

13. What protein in translation is responsible for translocation?

____

14. What is on the 5' end of eukaryotic mRNAs that is not on the 5' end of prokaryotic mRNAs? ________________

15. Draw the Fischer form of D-glucose and its enantiomer (6 points)

Glucose = ________________ Enantiomer = ________________

16. When cells are going through fermentation, what molecule are all of them trying to produce? ________________

17. Define feedback inhibition ________________

18. Besides glycogen, what other molecule is the substrate of glycogen synthase? _______________

19. What enzyme of gluconeogenesis is inhibited by F2,6BP?

____

20. Besides acetyl-CoA, what is the only other 2 carbon molecule in the glyoxylate cycle? _______________

21. What enzyme catalyzes the 'big bang' of glycolysis?

____

22. Which molecule of the electron transport system is referred to as the 'traffic cop'?

____

23. Name the molecule that directly donates two carbons to the growing fatty acid chain. _______________

24. What enzyme of the cholesterol biosynthesis pathway is feedback inhibited? _______________

25. Besides palmitoyl-CoA, what other molecule listed in class is a precursor of sphingolipids? _______________

26. What molecules are substrates for ribonucleotide reductase (answer must be precise) _______________

27. When you develop gout, what molecule is precipitating in your cells? _______________

28. Name an anti-cancer drug discussed in class that mimics folate _______________

Section III – Problems/Long Answer

1. One liter of a buffer from the blood of Modest Mouse singer Isaac Brock is found to have a pH of 7.2. The pKa of this buffer is 7.4. Does the buffer have more salt or more acid? (1 point)

Use the Henderson-Hasselbalch equation to justify your answer. Your answer MUST be based in the Henderson-Hasselbalch equation. Other answers will automatically be wrong. (7 points)

2. Describe the sequence of events involved in the elongation of translation in prokaryotes. Name the relevant proteins, RNA molecules, and structures described in class and the functions they perform. (9 points)

3. A reaction has twice as much product as reactant at equilibrium. What is the value of the $\Delta G^{\circ\prime}$? (I am not looking for a number here) (2 points)

Which direction will the reaction go when there are equal amounts of reactant and product? (Your answer MUST be based in the ΔG equation) (6 points)

4. Compare and contrast electron transport and oxidative phosphorylation with the light reactions of photosynthesis with respect to electron sources, electron acceptors, movement of protons, and any products of these reactions.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Exam 1 Key (BB 450/550)

Section I – Short answer - The questions below can generally be answered in 15 words or less. While you will not be required to use 15 words or less, excessively long answers will be scrutinized closely. Each correctly filled in blank below will be awarded three points, except as noted. (Answers in RED)

1. You have an acetate buffer. You add sodium acetate (salt) to it. What happens to the pH?

   The pH increases

2. You are examining a 2D gel. What was the name of the first technique that was used to perform this technique?

   Isoelectric focusing

3. You want to make the smallest polypeptide you can that will have the maximum positive charge without using the same amino acid twice. Give a sequence of such a polypeptide that you would make

   Many possibilities - one is His-Lys-Arg

4. Name all of the amino acids that cannot exist as zwitterions

   There are none

5. You have an acetate buffer. You manage to magically destroy half of the sodium acetate in it without converting it into anything else. What happens to the pH of the resulting solution?

   pH decreases

6. Tell precisely/completely what one joins together to make a peptide bond. Structures not necessary or desired.

   Link the carboxyl end of one amino acid to the amino end of the next one

7. Scurvy arises as a result of the structural weakness of what compound in the body?

   Collagen

8. Name the categories described in class that the following amino acids are grouped in

   Leucine = Aliphatic

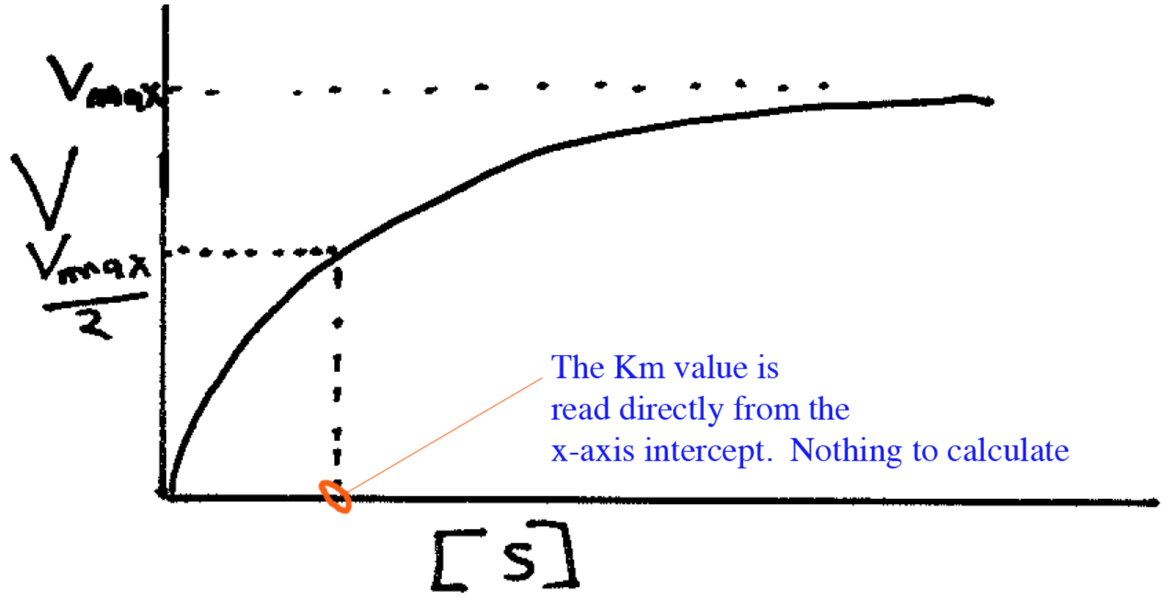
   Cysteine = Sulfhydryl

   Asparagine = Carboxyamide

9. What chemical named in class can one use to reduce a covalent bond between two cysteine side chains?

Mercaptoethanol or dithiothreitol

10. A scientist studies the kinetic behavior of an enzyme and obtains the following plot. Clearly mark the point on the graph you would use to calculate Km (2 points) and tell precisely how you would calculate Km from the value of this point (2 points).



The Km value is read directly from the x-axis intercept. Nothing to calculate

11. Velocity of a car is given in miles per hour. How is the velocity of an enzymatic reaction given? (must be precise)

[Product]/time

12. What is the name of the technique described in class uses beads with tiny "tunnels" in them?

Gel (molecular) exclusion chromatography

13. Name all of the levels/types of protein structure stabilized by hydrogen bonds

Secondary, tertiary, quaternary

Section 2 – Calculations - For each of the problems in this section, ORGANIZE and LABEL your calculations clearly. No partial credit will be given without clearly labeled calculations.

1. You have 1L of a 0.6M buffer at maximum buffering capacity. When you add 0.1 moles of HCl to the buffer (no volume change), the pH is 9.4. Show how you would calculate the pKa of the buffer.

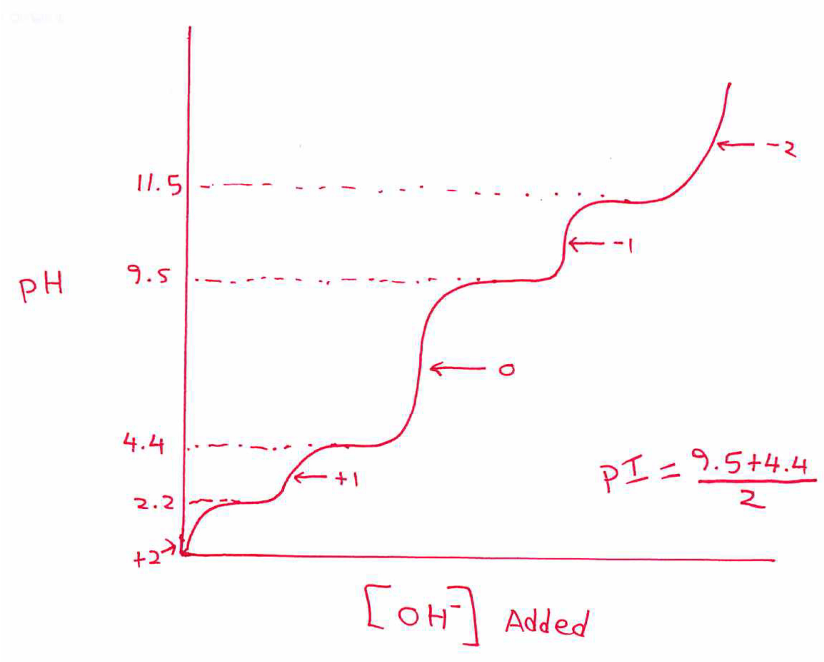
at maximum buffering capacity, [S] = [A]. This means 0.3 moles of each in 1L. Adding 0.1 moles of HCl converts 0.1 moles of S into A, resulting in 0.4 moles of S and 0.2 moles of A. Consequently, the pKa can be calculated as

pKa = 9.4-log{0.4/0.2}

2. A scientist has the polypeptide shown below at pH = 0 and begins adding NaOH to it.

glycine-arginine-leucine-aspartic acid-methionine-leucine

a. Using the pKa values from the first page of the exam, draw the titration curve (with properly labeled axes) for this molecule up to pH = 14.



b. Indicate the charge on the polypeptide at each relevant place on the graph

c. Show how to calculate the pI of this polypeptide

Section 3 – Explanations –For each question, provide a brief explanation of the phenomenon. Long rambling answers that are not to the point will lose points, even if they contain part of the correct answer.

1. I named at least four molecules/atoms/ions that affect hemoglobin's structure upon binding to it. Name each molecule/atom/ion and describe precisely where it binds on hemoglobin and how it affects oxygen affinity (20 points)

Protons - binds on amine side chains. Changes hemoglobin's structure to favor release of oxygen

2,3 BPG - binds in the "donut hole". Favors T-state - release of oxygen

Carbon dioxide - binds protonated histidines. Favors release of oyxgen

Oxygen - binds iron in heme group. Favors R-state - more binding of oxygen

2. Your friend is studying an enzyme in the presence of a non-competitive inhibitor and says that it gives a value of K_{cat} different than the same enzyme without the non-competitive inhibitor. Another friend says that it should have the same K_{cat} as the uninhibited reaction. The head of the lab walks up and says that both statements can be correct, depending on how you calculate K_{cat}. What is the head of the lab thinking? (10 points)

In the case of the first friend, he/she is only considering the total amount of enzyme. The second friend is only considering the amount of enzyme that is actually active.

Since the same concentration of product is produced in both cases, the ratios

[Product]/[Total Enzyme = active + inactive]

and

[Product]/[Total Enzyme = active]

will be different, even though the same concentration of product exists. Thus, either may be right, depending on the concentraiton of enzyme used.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Exam 2 Key (BB 450/550)

Section I – Short answer - The questions below can generally be answered in 15 words or less. While you will not be required to use 15 words or less, excessively long answers will be scrutinized closely. Each correctly filled in blank below will be awarded three points, except as noted.

1. What is the difference between the DNA sequence recognized by a restriction enzyme and the DNA sequence recognized by its corresponding methylase?

No difference

2. Carbonic anhydrase is more active at pH 8.5 than it is at pH 7. Precisely what is favored at the higher pH that causes this?

Deprotonation of water

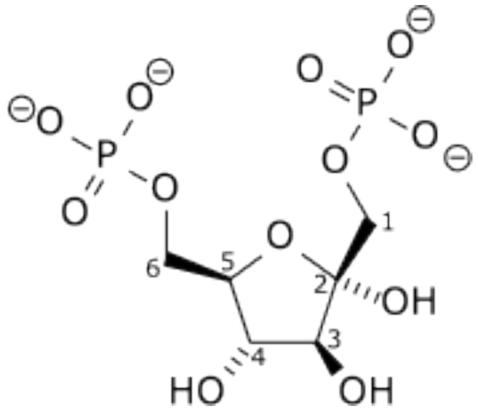
3. I talked about at least three different ions that could act as nucleophiles in enzyme catalysis. Draw three of these (hint – be sure to draw the form of each ion that acts as the nucleophile)

$–O^-$ / HO^- / $–S^-$

4. Only one of the allosteric effectors of ATCase favors the T-state. Which one?

CTP

5. Draw the structure of the first glycolysis intermediate containing two phosphates.



6. Precisely what chemical reaction on what molecule/compound/ enzyme does warfarin directly inhibit (four points)?

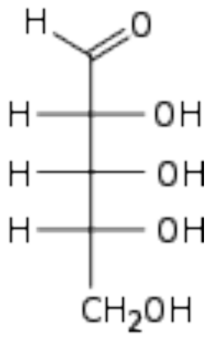
Carboxylation of glutamate side chains on prothrombin

7. Define the term 'anomers'

Two sugar molecules differing in structure

only at the anomeric carbon

8. Draw the Fischer structure of and name the only pentose you are responsible for knowing the structure of (no partial credit)



The sugar is ribose

9. What is the name of the only enzyme of glycolysis that catalyzes a redox reaction?

Glyceraldehyde-3-phosphate dehydrogenase

10. In the catalytic action of serine proteases, what must happen to move the catalytic triad closer together to create the alkoxide ion and start the catalytic process?

Binding of the proper substrate at the S1 site

11. Where in the cell are O-linked glycoproteins made?

Golgi apparatus

12. What are the name(s) of the second/third messenger(s) for the pathway involving phospholipase C?

2nd = IP_3

3rd = Ca^{++}

13. Name a compound described in class that is an inhibitor of BCR-ABL

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Section 2 – Calculations - For each of the problems in this section, ORGANIZE and LABEL your calculations clearly. No partial credit will be given without clearly labeled calculations. (15 points each)

1. A reaction is at equilibrium at a temperature of 300K with a [Reactant] = .4M and [Product] = 1.0M. Using as much of this information as possible, write an equation to calculate the ΔG when there are equal amounts of the two (15 points)

$$0 = \Delta G°' + 300RLn[1/.4], \text{ so } \Delta G°' = -300RLn[1/.4]$$

then

$$\Delta G = -300RLn[1/.4] + 300RLn[1/1],$$

but $Ln[1/1] = 0$, so

$$\Delta G = -300RLn[1/.4]$$

2. The reaction below meets the needs of the body's muscles remarkably well. Explain how the G for this reaction varies according to whether one is exercising or resting and how that provides the muscles with what they need to keep the body functioning. (15 points)

Creatine + ATP <=> Creatine phosphate + ADP

($\Delta G°' = + 12$ kJ/mol)

I use 'C' here to mean creatine and 'CP' to mean Creatine phosphate

1. When exercising, [ATP] falls and [ADP] rises, thus the Ln term of the ΔG equation,

Ln{[CP][ADP]/[C][ATP]} becomes more positive, moving the equilibrium to the left, favoring the production of ATP when it is needed.

2. At rest, the opposite happens, so the Ln term becomes more negative, favoring storing of energy in the form of CP

Section 3 – Explanations –For each question, provide a brief explanation of the phenomenon. Long rambling answers that are not to the point will lose points, even if they contain part of the correct answer.

1. You have four friends. Two are born without alpha-1-antitrypsin and two are born with a normal alpha-1-antitrypsin. Their circumstances are below:

A. No alpha-1-antitrypsin - smokes
B. No alpha-1-antitrypsin - doesn't smoke
C. Regular alpha-1-antitrypsin - smokes
D. Regular alpha-1-antitrypsin - doesn't smoke

Predict the relative tendency to have emphysema you would expect for each of the friends in the comparison below based on class discussion. Justify your answer at the end of the problem. (15 points)

a. Compare A to B (is emphysema in A > B, A<B, or A=B)
b. Compare C to D (is emphysema in C > D, C<D, or C=D
c. Compare B to D (is emphysema in B > D, B<D, or B=D)

a. A = B

b. C > D

c. B > D

2. You are walking home one night when out of the bushes jumps your biochemistry professor singing loudly and very off key. This scares you terribly, so you start secreting the same hormone you would if a grizzly bear were chasing you. Name the hormone and show/describe the entire signaling pathway described in class that it activates. (15 points)

The hormone is epinephrine

The signaling pathway goes as follows

Epinephrine binds to the beta-adrenergic receptor, activating it. The beta-adrenergic receptor activates a G protein, favoring it dumping GDP and loading GTP. The activated G protein activates adenylate cyclase, which makes cAMP. cAMP activates protein kinase A, which in turn favors production of glucose by (ultimately) stimulating enzymes that breakdown glycogen and activate gluconeogenesis

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Final Exam Key (BB 450/550)

Section I – Short answer - The questions below can generally be answered in 15 words or less. While you will not be required to use 15 words or less, excessively long answers will be scrutinized closely. Each correctly filled in blank below will be awarded three points, except as noted.

1. One method of separating proteins described in class combines two different separation techniques. Name the overall method and the names of the two separation techniques.

Overall method = 2D gel electrophoresis.

The two techniques are isoelectric focusing and SDS-PAGE

2. Name all of the amino acids described in class that can be phosphorylated

Tyrosine, serine, threonine (histidine can also be phosphorylated, but it wasn’t described in class)

3. Describe or illustrate (precisely) below the reaction that mercaptoethanol stimulates

Mercaptoethanol reduces disulfide bonds to sulfhydryl bonds

4. Velocity of a car is given in miles per hour. How is the velocity of an enzymatic reaction given? (must be precise)

[Product]/Time

5. Name three forces stabilizing quaternary interactions

hydrogen bonds, ionic bonds, disulfide bonds,

hydrophobic bonds

6. A deficiency of what dietary molecule causes scurvy?

Vitamin C

7. Name the only molecule described in class that competes with oxygen for the binding site on hemoglobin

Carbon monoxide

8. To what atom does oxygen bind in hemoglobin?

Fe^{++}

9. Precisely where on ATCase does aspartate bind?

Active site

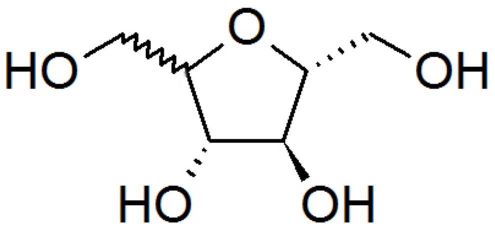
10. What is the name of the enzyme in glycolysis that produces an intermediate that affects hemoglobin's affinity for oxygen?

Phosphoglycerate mutase

11. Name the only enzyme in glycolysis that is regulated both allosterically and by covalent modification

Pyruvate kinase

12. Draw the Haworth structure for the only hexose given in class for which you must know the furanose form



13. What is the name of the enzyme that catalyzes the breakdown of blood clots?

Plasmin

14. In the catalytic action of serine proteases, what must happen to move the catalytic triad closer together to create the alkoxide ion and start the catalytic process?

Proper substrate must bind at the S1 site

15. Gleevec inhibits Bcr-Abl. What enzymatic reaction does this protein catalyze?

Phosphorylation of tyrosine

16. What bond is broken by the nucleophilic attack in the fast step of the catalytic mechanism of serine proteases?

Peptide bond

17. Name three first messengers described in class

ephinephrine, glucagon, insulin

18. A friend tells you that a new buffer has been discovered in which there is more salt than acid when the pH is the same as the pKa. Using math, explain why this is true or false. (Your answer must also say "TRUE" or "FALSE")

False

pH = pKa + Log {[Salt]/[Acid]}.

Thus if pH = pKa, then Log {[Salt]/[Acid]} must be zero, but the ratio of [Salt]/[Acid] is not equal to one, so the statement must be wrong.

19. Kevin Ahern's hypothesis of why Americans are becoming more and more obese says it is due to the bypassing of an important enzymatic reaction. What is the name of this enzyme?

Phosphofructokinase (PFK)

20. What molecule is synthesized in animals in order to make NAD^+ when oxygen levels are low?

Lactate

21. In utilization of galactose, it is first phosphorylated and added to a molecule that results in the release of glucose-1-phosphate. To what molecule is the phosphorylated galactose attached?

UDP

22. Name a transcription factor made/activated in cells when oxygen is lacking.

HIF-1

23. Name a molecule that activates an enzyme in feed-forward activation.

F1,6BP (Fructose-1,6-bisphosphate)

24. Name the only enzyme I described in class that is used both in the breakdown of glycogen pathway and the synthesis of glycogen pathway

Phosphoglucomutase

25. What does it take to convert glycogen phosphorylase a from the T state to the R state?

Nothing. It automatically flips

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Section 2 – Calculations - For each of the problems in this section, ORGANIZE and LABEL your calculations clearly. No partial credit will be given without clearly labeled calculations. (12 points each)

1. You have 1L of a 0.6M buffer with twice as much salt as acid. After you add .1 moles of HCl (no volume change), the pH is 8.32. Write an equation to calculate the pH before the HCl was added.

To start, you have 0.4 moles salt and 0.2 moles acid

Upon adding the HCL, you have 0.3 moles of salt and acid

Thus, the pH at this point is the pKa, as well.

Consequently, before the HCl was added, the pH is given as follows:

pH = 8.32 + Log(0.4/0.2)

2. A reaction starts with 5 times as much reactant as product, but at equilibrium, it only has 3 times as much reactant as product.

a. Write an equation to show how to calculate ΔG°' (3 points)

$$0 = \Delta G^{\circ\prime} + RTLn[1/3].$$

$$\Delta G^{\circ\prime} = -RTLn[1/3]$$

b. Write an equation to show how to calculate ΔG at the very beginning (3 points)

$$\Delta G = -RTLn[1/3] + RTLn[1/5]$$

c. Show how you would determine the sign of the ΔG°' (3 points)

Since 1/3 <1, the log term must be negative,

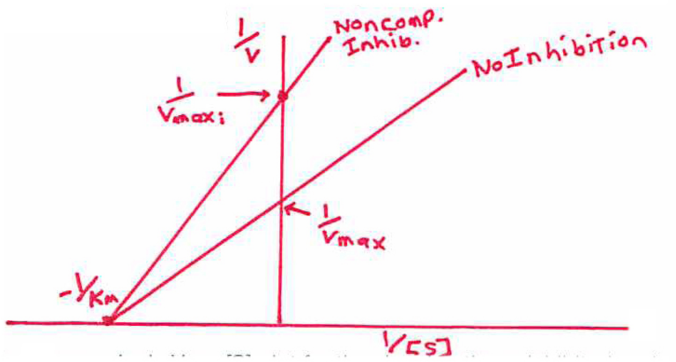
so the ΔG°' must be positive

d. What was the sign of ΔG at the start of the reaction? Why? (3 points)

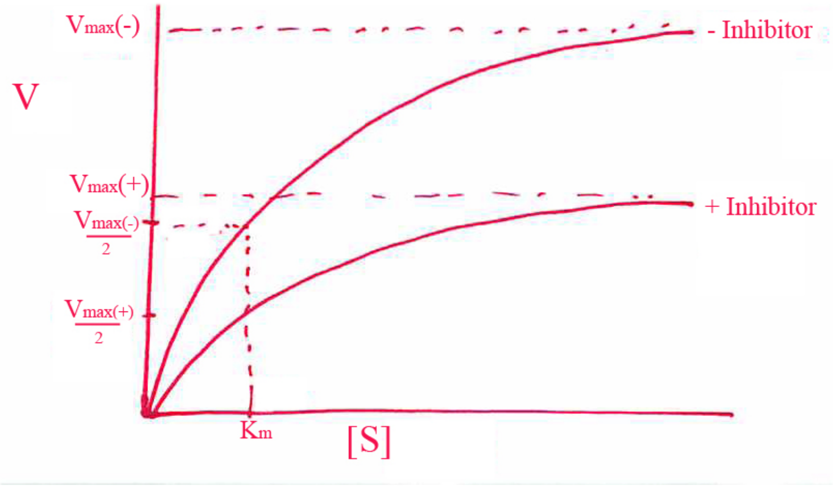
Since the reaction goes from 5 times as much reactant as product to only 3 times as much reactant as product, the reaction must have run forwards. Thus, the ΔG was negative then.

3. An analysis of an enzymatic reaction is performed in the presence and absence of an inhibitor. The inhibitor appears only to reduce the V_{max} of the enzyme.

a. Draw a single Lineweaver Burk plot showing the uninhibited and the inhibited reaction. Clearly label all relevant features on the graph. (5 points)



b. Draw a single V vs. [S] plot for the showing the uninhibited and the inhibited reaction. Clearly label all relevant features on the graph. (5 points)



c. What type of inhibition is occurring? (2 points)

Non-competitive inhibition

Section 3 – Explanations –For each question, provide a brief explanation of the phenomenon. Long rambling answers that are not to the point will lose points, even if they contain part of the correct answer.

1. Regulation of glycolysis and gluconeogenesis is complex. It involves allosteric interactions, covalent modifications, and control of enzymes that make/break down molecules that affect the pathway.

a. Name the enzymes regulated in each pathway (7 points)

Glycolysis - hexokinase, PFK, pyruvate kinase

Gluconeogenesis - pyruvate carboxylase, PEPCK, F1,6BPase, G6Pase

b. Name one allosteric activator and one allosteric inhibitor of the reciprocally regulated enzmes above(4 points)

PFK - F2,6BP, AMP activators. ATP, citrate inhibitors

F1,6BPase - Citrate activator, F2,6BP, AMP inhibitors

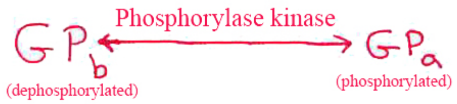
c. Name the most important allosteric regulator of the two pathways and show precisely how its synthesis and breakdown is regulated. (6 points)

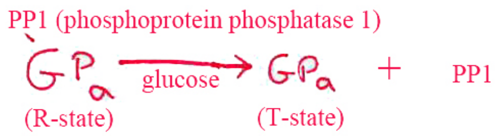
Most important regulator is F2,6BP.

Its synthesis is activated by dephosphorylation of FBPase2/PFK2 by phosphoprotein phosphatase (ultimately activated by insulin).

Its breakdown is activated by phosphorylation of FBPase2/PFK2 by Protein Kinase A (ultimately activated by epinephrine)

2. Glycogen phosphorylase is regulated allosterically and by an enzyme it carries around with it. Draw a scheme illustrating all of the ways in which glycogen phosphorylase is regulated and also a scheme showing how the enzyme it carries can affect the enzyme. (10 points total)





GP_b inhibited by ATP, G6P
GP_b activated by AMP
GP_a inhibited by glucose

$GP_a \xrightarrow[\text{PP1}]{} GP_b$
$GS_b \xrightarrow[\text{PP1}]{} GS_a$

3. We have talked this term about three broad ways in which enzyme activity can be regulated. Name each way. Name one enzyme regulated each way. Give detail as appropriate for each means of regulation. If external molecules or modifications are relevant, your answer must include them and a description must include the effect the molecules or modifications have on each enzyme. (12 points)

A. Allosteric regulation - several examples. One is ATCase, which is allosterically activated by ATP and allosterically inhibited by CTP

B. Covalent modification - several examples. One is glycogen synthase, which is inactivated by phosphorylation by protein kinase A and activated by dephosphorylation by phosphoprotein phosphatase.

C. Control of whether an enzyme is made or not. A good example is PEPCK in gluconeogenesis. It is not made in many tissues, but is made in liver and kidney cells where gluconeogenesis is active

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Exam 1 Key (BB 451/551)

Section I: (20 points total) The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive four points if you circled 'b','c', and 'd'. You would receive one point if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings.

This is basically a True/False type of questioning. True answers below are marked in RED

Practice question #A: Oregon State University

A. is a Peruvian factory
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world.

1. With respect to the citric acid cycle,
   A. succinate dehydrogenase requires FAD for catalysis
   B. it includes two decarboxylations not found in the glyoxylate cycle
   C. NADH is not used
   D. it uses oxygen in one or more of its enzymatic reactions

2. With respect to respiratory control,
   A. cyanide will stop oxidative phosphorylation first
   B. exercising will favor electron transport
   C. resting will favor decrease in ADP
   D. 2,4 DNP (miracle diet drug) will increase NADH and temperature

3. With respect to membrane transport,
   A. lactose permease is an anti-port
   B. it requires ATP
   C. it requires diffusion through the membrane
   D. it cannot be electroneutral if sodium is involved

4. With respect to electron transport,
   A. electrons get pumped into the intermembrane space
   B. oxygen is the terminal proton acceptor
   C. the Q cycle occurs in Complex III
   D. 2,4 DNP will increase oxygen consumption

5. In tightly coupled mitochondria,
   A. increasing oxygen will increase the proton gradient
   B. lack of NAD$^+$ will ultimately reduce electron flow
   C. uncoupling protein (UCP) will reduce ATP production
   D. the citric acid cycle will run fastest when electron transport runs fastest

Section II: (50 points total) Each sentence below in this section is missing a word or phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded two points.

1. Name the five carbon intermediate in the citric acid cycle

alpha-ketoglutarate

2. Which enzyme of the citric acid cycle catalyzes a reaction pulls a reaction preceding it?

citrate synthase

3. Which enzyme of the citric acid cycle requires lipoamide?

alpha-ketoglutarate dehydrogenase

4. Which enzyme of the citric acid cycle catalyzes the only oxidation that doesn’t produce NADH?

succinate dehydrogenase

5. What citric acid cycle intermediate can be readily made from glutamic acid in an anaplerotic reaction?

alpha-ketoglutarate

6. How many oxaloacetates are produced per turn of the citric acid cycle?

one

7. Name a poison described in class that reacts with a citric acid cycle coenzyme

arsenic

8. Which class of membrane-related proteins would be the most likely to have hydrophobic amino acids on its exterior?

integral

9. If one membrane protein pump were missing from the cell, neural transmission as described in class would not be possible. What pump is that?

Na^+/K^+ ATPase

10. What is the basis for exclusion of potassium from a sodium channel?

size

11. What is the basis for exclusion of sodium from a potassium channel?

energy of hydration/dehydration

12. Active transport always has at least one mechanistic difference from passive transport. It is the reason that active transport requires energy. What is that difference?

In active transport, at least one molecule is being moved against a concentration gradient

13. What is the driving force for passive transport?

diffusion

14. What is the definition of a symport?

a membrane transport system that moves two molecules in the same direction across the membrane

15. You remove digitoxigenin from a heart cell that had been treated with it. What happens to the concentration of sodium outside of the cell?

increases

16. In the Q cycle, what takes electrons away from Complex III?

cytochrome C

17. Complex V has a rotating complex that make ATP. It has three different configurations. What is the form of the configuration where ATP is released?

Open (O)

18. You treat a mitochondrion with 2,4 DNP and cyanide. What happens to oxygen consumption?

Little or nothing

19. A mitochondrion is treated with 2,4 dinitrophenol (2,4 DNP). What happens to its oxygen consumption?

increases

20. The mitochondria in a cell are tightly coupled. The citric acid cycle is running furiously. What normal explanation (not exotic or unusual) makes the most sense about what this cell is doing to cause this?

Cell is burning ATP rapidly

21. If one wanted to make a photosynthetic fish, as described in class, what protein would you need to do it?

bacteriorhodopsin

22. Describe the numbering system for an omega fatty acid. Which carbon is number one?

methyl carbon

23. What is the name of the category of ATP-using transport proteins that involve a phosphoaspartate?

P-type ATPases

24. What is the charge on a copper atom in Complex IV after it has accepted an electron?

+1

25. If you wanted to raise the T_m of a membrane, how would you alter the chemical composition of its fatty acids?

make them longer and/or more saturated

Section III: (30 points total) Matching.

T	1. Na/K ATPase	A. Can stop oxygen consumption indirectly
M	2. Isocitrate	B. Limiting for people not exercising
U	3. Brown fat	C. Produced by superoxide dismutase
R	4. Acetaldehyde	D. Glyoxylate cycle enzyme requirement
L	5. Decarboxylation	E. Carries electrons in pairs
G	6. Sphingomyelin	F. Five carbon intermediate
D	7. Malate synthase	G. Phosphorylated membrane component
A	8. Antimycin A	H. Neurotransmitter described in class
C	9. Oxygen	I. Neurotransmitter holder
B	10. ADP	J. Complex IV inhibitor
C	11. H_2O_2	K. Citrate synthase byproduct
E	12. Coenzyme Q	L. Process that does not occur in glyoxylate cycle
I	13. Synaptic vesicle	M. Six carbon intermediate
Q	14. Malate	N. Symport
V	15. Thiamine pyrophosphate (TPP)	O. Blocked by tetrodotoxin
		P. Produced by citrate synthase
		Q. Gets oxidized after being shuttled
		R. Ethanol precursor
		S. Produced by oxidative phoshorylation
		T. Electro-genic
		U. Can become uncoupled
		V. Carries a two carbon group

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Exam 2 Key (BB 451/551)

Section I: (20 points total) The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive four points if you circled ‘b’,’c’, and ‘d’. You would receive one point if you circled ‘a’ and ‘b’. You would receive no points if you circled only ‘a’. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings.

These are basically True/False question. True answers are show in RED

Practice question #A: Oregon State University

A. is a Peruvian factory
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world

1. With respect to cholesterol metabolism,
    A. it uses palmitoyl-CoA and serine
    B. it includes an intermediate of squalene
    C. ATP is not used
    D. it is a precursor to vitamin D, bile acids, and steroid hormones

2. With respect to fatty acid metabolism,
    A. it starts with oxidation of medium chain fatty acids in peroxisomes
    B. oxidation of saturated fatty acids involves *cis* intermediates
    C. enoyl-CoA isomerase is not needed for oxidation of saturated fatty acids
    D. ketone bodies use the last enzyme of fatty acid synthesis

3. With respect to prostaglandins,
    A. ibuprofen is a COX-1 inhibitor
    B. steroids inhibit COX-2 enzymes
    C. they are usually produced from thromboxanes or leukotrienes
    D. PLA-2 inhibits the action of aspirin

4. With respect to nucleotide metabolism,
    A. deoxyribonucleotides are made from ribonucleoside diphosphates
    B. PRPP amidotransferase balances relative amounts of pyrimidines
    C. NDP Kinase (NDPK) converts ADP to dADP
    D. dUTPase converts dUTP to dTTP

5. With respect to DNA replication,
    A. the beta clamp is responsible for making DNA Polymerase III more processive
    B. helicase forms double helices at the rate of 6000 rpm
    C. Okazaki fragments are only made in the 5’ to 3’ direction
    D. lagging strand replication occurs in the 3’ to 5’ direction

Section II: (50 points total) Each sentence below in this section is missing a word or phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below, except as noted, will be awarded two points.

1. What is the name of the transcription factor that helps control synthesis of HMG-CoA reductase?

SREBP

2. I named four general mechanisms for regulating HMG-CoA reductase. Name three of them (1 point each)

control of synthesis, allosterism, covalent modification, half-life (time to destruction)

3. What group of molecules has their synthesis stopped by aromatase inhibitors?

estrogens

4. I mentioned three factors to consider for controlling the level of cholesterol in the body. Name them (1 point each)

diet, synthesis, storage/recycling

5. What is the name of the cellular structure missing in familial hypercholesterolemia?

liver receptor for LDLs

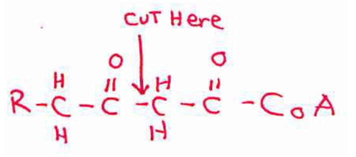
6. What is the name of the last product of beta oxidation of a fatty acid with an odd number of carbons?

propionyl-CoA

7. Name the only regulated enzyme in either fat or fatty acid breakdown

triacylglycerol lipase

8. Draw the structure that the enzyme thiolase would cut



9. Describe accurately in words what enoyl-CoA isomerase catalyzes

Conversion of *cis* bonds in fatty acids between carbons 3-4 into *trans* bonds between carbons 2-3

10. Name the enzyme that catalyzes the first reaction in the pathway leading to ketone bodies

thiolase

11. Name the molecule produced by catalysis by the only regulated enzyme of fatty acid biosynthesis

malonyl-CoA

12. Name the molecule that shuttles the precursor of fatty acid synthesis to the cytoplasm

citrate

13. How does carbamoyl phosphate synthetase protect its intermediate product from water?

by moving it through a tunnel

14. What is the name of the enzyme that balances purines and pyrimidines in *de novo* ribonucleotide synthesis?

ATCase

15. What molecule is the energy source for *de novo* synthesis of AMP?

GTP

16. What is the name of the molecule that is the branch point in *de novo* purine ribonucleotide synthesis?

Inosinate (IMP)

17. Write out the reaction catalyzed by adenylate kinase

AMP + ATP <=> 2ADP

18. What atom does RNR (ribonucleotide reductase) catalyze the removal of?

Oxygen

19. What is the name of the electron source for fatty acid biosynthesis?

NADPH

20. What is the name of the electron acceptor in the first step of fatty acid oxidation?

FAD

21. Name the form of DNA discovered by Rosalind Franklin

A-form

22. Name the enzyme necessary for joining the DNAs of Okazaki fragments

DNA ligase

22. What is the name of the first protein to bind to the *E. coli* DNA replication origin?

DNA-A

23. Precisely what does telomerase use as a template to make telomeres?

an RNA that it carries

24. What enzymatic activity is present in DNA polymerase I and III, but lacking in reverse transcriptase of HIV?

Proofreading (3’ to 5’ exonuclease activity)

25. Give the ratio that determines whether or not a DNA is relaxed or not (note – I am looking for the units, not a number)

bp/turn

Matching

U 1. dnaB
M 2. Guanosine
L 3. Gyrase
I 4. Arachidonic acid
B 5. LDL
P 6. Serine
N 7. CDP
F 8. Cholesterol
V 9. dATP
R 10. S-Adenosyl Methionine (SAM)
S 11. HMG-CoA
A 12. Lipase
J 13. Ganglioside
E 14. ACP
T 15. Glycerol

A. Necessary for fat digestion
B. Lipoprotein complex
C. Palmitate precursor
D. Requires vitamin B_{12}
E. Replaces CoA of malonyl-CoA
F. Feedback inhibitor of long pathway
G. ATCase inhibitor
H. Lacking in Lesch-Nyhan syndrome
I. Leukotriene precursor
J. Sphingolipid
K. Prostaglandin product
L. Topoisomerase
M. Nucleoside
N. Found in activated intermediates
O. Covalent modification product
P. Necessary for sphingolipid synthesis
Q. Glycerophospholipid
R. Methyl donor
S. INSIG target
T. Can be made into glucose
U. Helicase
V. Allosteric inhibitor of ribonucleotide reductase

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Final Exam Key (BB 451/551)

Section I: (40 points total) The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive four points if you circled 'b','c', and 'd'. You would receive one point if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings.

These are basically True/False questions. True answers are marked below in RED

Practice question #A: Oregon State University

A. is a Peruvian factory
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world.

1. With respect to the citric acid cycle,

A. GDP is required
B. lipoic acid has no role
C. oxygen is not a substrate in the reactions of the cycle
D. oxidative decarboxylation does not occur

2. With respect to movement of compounds across biological membranes,

A. active transport requires ATP
B. water cannot readily cross membranes
C. a proton gradient is required
D. protons generally require a protein to move across membranes

3. In tightly coupled mitochondria,

A. oxidative phosphorylation will speed up to make up for an oxygen deficit
B. low ADP levels will have the same overall effect as low oxygen levels
C. increasing intermembrane space pH will reduce ATP production
D. cyanide will reduce both ATP production and the proton gradient

4. With respect to the Q cycle,

A. it explains how electrons arrive in pairs, but are converted to moving singly
B. it involves coenzyme Q in three different forms
C. Cytochrome C carries electrons to complex III
D. each QH_2 sends electrons to two different things

5. With respect to fatty acid oxidation,

A. it occurs in the cytoplasm and the peroxisomes
B. it produces $FADH_2$ and NADH
C. it only occurs on fatty acids shorter than 16 carbons
D. it occurs when the fatty acid is bound to ACP

6. With respect to fatty acid synthesis,

- A. it is allosterically activated by palmitate/palmitoyl-CoA
- B. it occurs in the cytoplasm and endoplasmic reticulum
- C. it uses malonyl-ACP
- D. it requires a carboxylation and a decarboxylation

7. With respect to prostaglandins,

- A. aspirin is a COX inhibitor
- B. inhibiting phospholipase A_2 will stop release of fatty acids from membranes
- C. they are precursors of cholesterol
- D. they are pain relievers

8. With respect to nucleotide metabolism,

- A. it uses ribonucleotide reductase to convert all dNDPs to dNTPs
- B. it uses CTP to make UTP
- C. it uses dTMP to make dUMP
- D. it uses DHFR to make dNDPs from NDPs

9. With respect to transcription,

- A. it occurs at a structure called a replication fork
- B. the coding strand is complementary to the RNA being made
- C. it makes RNA in the 3’ to 5’ direction
- D. it requires a ribosome

10. With respect to gene expression,

- A. attenuation involves early transcriptional termination
- B. the lac repressor binds to DNA when it binds allo-lactose
- C. CAP binds to DNA when it binds to cAMP
- D. IRE-BP binds to DNA when it does not bind iron

Section II: (80 points total) Each sentence below in this section is missing a word or phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded two points.

1. What coenzyme named in class does arsenate react with?

lipoamide (lipoic acid)

2. Which enzyme of the citric acid cycle catalyzes a reaction that produces $FADH_2$?

succinate dehydrogenase

3. What is the definition of an antiport?

a membrane transport protein that moves two molecules in opposite directions across the membrase

4. Which enzyme of the citric acid cycle catalyzes a reaction that pulls the one preceding it?

citrate synthase

5. What amino acid is alpha-keto-glutarate readily converted to in an anaplerotic reaction?

glutamic acid

6. Name one enzyme unique to the glyoxylate cycle

malate synthase or isocitrate lyase

7. You take a heart cell that was treated with digitoxigenin and remove the digitoxigenin. What happens to the concentration of calcium outside of the cell?

increases

8. Name a specific sphingolipid named in class that contains a phosphate

sphingomyelin

9. Name the class of lipid described in class that contains complex carbohydrates

gangliosides

10. A gradient of which ion is used as an energy source to remove calcium ions from heart cells?

sodium

11. What is the criterion we use to determine if a transport mechanism is active versus passive?

active transport always has at least one molecule/ion moved against a concentration gradient

12. In what class of membrane transporters does phosphoaspartate appear?

P-type ATPases

13. A cell (and its mitochondria) are treated with 2,4 dinitrophenol (2,4 DNP). What happens to the citric acid cycle in this cell?

speeds up

14. Name one of the two isoprenes described in class

isopentenyl pyrophosphate or dimethylallyl pyrophosphate

15. Complex V has a rotating complex that makes ATP. It has three different configurations. What is the form of the configuration where ATP is released?

the O configuration

16. Describe precisely the catalytic activity of DNA Polymerase I that removes RNA primers.

5’ to 3’ exonuclease

17. Precisely what does telomerase copy to make a telomere?

a strand of RNA it carries

18. How does one completely inhibit PRPP amidotransferase?

with high amounts of both AMP and GMP

19. What is the name of the molecules that donate single carbons in nucleotide metabolism (hint – I'm not looking for carbon dioxide or bicarbonate) ?

folates

20. What molecule does aspirin prevent from being acted on by COX?

arachidonic acid

21. In fatty acid synthesis, describe the chemical step after deyhdration (all I need is a name describing the chemical reaction)

hydrogenation

22. Name the enzyme that catalyzes conversion of fatty acids with a *cis* double bond between carbons 3/4 to a *trans* double bond between carbons 2/3?

enoyl-CoA isomerase

23. What causes gout?

overproduction of uric acid arising from purine catabolism

24. Where does one find acyl-CoA dehydrogenases that act on fatty acids of 20 carbons?

peroxisomes

25. Name a covalent modification of an enzyme in fatty acid metabolism and the effect of that covalent modification

Enzyme modified = acetyl-CoA carboxylase
Modification = phosphorylation / dephosphorylation
Effect of modification = reduce activity / increase activity

26. Name the enzyme used in DNA footprinting

DNAse

27. Name the specific catalytic entity that forms phosphodiester bonds between ribonucleotides.

RNA Polymerase

28. Name the specific catalytic entity that forms peptide bonds between amino acids in bacteria

23S rRNA

29. Define the term "template strand"

the strand of DNA that is "read" by RNA Polymerase during transcription. It is complementary to the RNA that is made

30. Name the protein described in class that unwinds DNA at 6000 rpm

helicase

31. In attenuation of the trp operon, what is the function of the structure in the mRNA that only forms when there is abundant tryptophan in the cell?

termination of transcription

32. What does tamoxifen prevent the estrogen receptor from interacting with?

co-activators

33. In elongation, which direction is the ribosome moving relative to the mRNA?

5’ to 3’

34. Name the 7TM of vision that is bound to Vitamin A

rhodopsin

35. Name the G protein involved in the sense of smell

G_{olf}

36. Describe the unusual bond in a lariat structure

5’ to 2’

37. Describe what rho does and how it does it

termination of transcription - “climbs” the RNA and “lifts” the RNA Polymerase off of the DNA it is copying

38. Precisely where is the wobble base of the anticodon found ?

5’ end of the anticodon

Section III: Matching. Each term on the left has a phrase or term on the right which best describes or matches it. Place the letter of the term/ phrase on the right in the blank before the term on the left that it best matches. Only one letter is appropriate in the blank. Note that there are more terms on the right than there are blanks, so not every term on the right has a best match. Terms on the right may be used once, more than once, or not at all. If we cannot read your writing or if you put two letters in any blank on the left, your answer will be counted wrong automatically. Each correctly matched pair is worth two points.

M 1. TPP
N 2. Intron
I 3. Transducin
P 4. Gyrase
U 5. dATP
H 6. Inducer
J 7. Thiolase
V 8. Statin
L 9. Active transporter
X 10. Tamoxifen
D 11. *Trans* fat
G 12. Okazaki fragments
T 13. Carnitine
E 14. Anti-codon
A 15. Ribozyme

A. Peptide bond catalyst(s)
B. Purine breakdown product(s)
C. Binds retinal
D. Hydrogenation byproduct(s)
E. tRNA component(s)
F. Spingolipid(s)
G. Lagging strand synthesis product(s)
H. IPTG
I. G protein(s)
J. Ketone body enzyme(s)
K. Prostaglandin enzyme(s)
L. Na/K ATPase
M. Pyruvate dehydrogenase complex component(s)
N. pre-mRNA component(s)
O. Reverse transcriptase(s)
P. Topoisomerase(s)
Q. Helicase(s)
R. Translation chain terminator(s)
S. Copper
T. Mitochondrial membrane crosser(s)
U. Ribonucleotide reductase inhibitor(s)
V. HMG-CoA reductase inhibitor
W. Fatty acid synthase end product(s)
X. Estrogen receptor binder(s)

Exam 1 Key (BB 350)

Section I: The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive eight points if you circled 'b','c', and 'd'. You would receive two points if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings. These are essentially true/fall questions. True answers are shown in RED.

Practice question #A: Oregon State University

A. is a factory in Portland
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world

1. With respect to hydrogen bonds,
    A. they are weaker than covalent bonds
    B. they are weaker than disulfide bonds
    C. they are what make peptide bonds
    D. water does not have to be involved

2. With respect to amino acids,
    A. glycine's R group is a hydrogen
    B. lysine's R group is more hydrophobic than leucine's
    C. methionine is the only one with a sulfur in the R group
    D. every one of them can exist as a zwitterion

3. With respect to enzymes,
    A. K_m is a velocity
    B. $V_{max}/2$ is the same as K_m
    C. K_{cat} varies with substrate concentration
    D. $K_{cat} = K_m$ /[enzyme]

4. With respect to information in the syllabus,
    A. there is no fixed grading scale
    B. grades are curved
    C. the grading scale is 90/80/70/60

Section II: 1. Each sentence below in this section is missing a word or short phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded three points. You MUST be precise in your definitions.

1. Describe the difference between a strong acid and a weak acid in solution.

A strong acid completely dissociates in an aqueous solution, whereas a weak acid does not

2. Name an amino acid that makes disulfide bonds

Cysteine

3. How does a fibrous protein differ from a globular protein?

Fibrous proteins have little tertiary structure, whereas globular proteins have much tertiary structure

4. A solution has a pH of 6. Write an equation for its hydroxide ion concentration

$[OH^-] = 10^{-8}M$

5. What is the name of the group in myoglobin that contains the iron?

heme

6. Define allosterism

when binding of a small molecule to an enzyme affects the enzyme's activity

7. You have a buffer solution with more salt than acid and the pH is 6. What can you say about the pK_a?

the pK_a is less than 6

8. Explain from an energetic perspective how enzyme catalysis works (one sentence should suffice).

Enzymes work by lowering the activation energy of the reactions they catalyze

9. What units does K_M have?

concentration (molarity, for example)

10. Name three separation techniques described in class

gel exclusion chromatogrphy, gel electrophoresis, PAGE, ion exchange chromatography, affinity chromatography

11. What name do we give to the molecule(s) that an enzyme catalyzes a reaction on?

substrate(s)

Section III – Problems/Long Answer

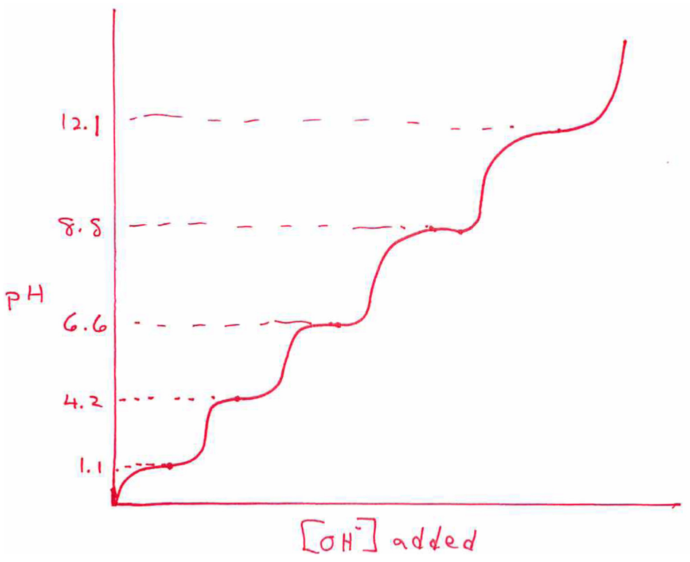
1. Explain at the molecular level why smokers breathe more heavily when exercising than non-smokers. (16 points)

Smokers have a higher concentration of 2,3 BPG in their bloodstream than non-smokers. 2,3 BPG binds to hemoglobin and favors the T state, thus reducing how much oxygen it can carry. As a result, smokers' blood can carry less oxygen than non-smokers' blood.

2. An exotic amino acid is discovered that has two amine groups and three carboxyl groups. The pKa values of the groups are below

Amino #1 = 8.8
Amino #2 = 12.1
Carboxyl #1 = 1.1
Carboxyl #2 = 4.2
Carboxyl #3 = 6.6

a. Draw and label CLEARLY a titration curve with all relevant values for this amino acid (12 points)



b. Calculate the approximate charge this amino acid would have at a pH of 5.5 (4 points)

Zero

3. For the following problem, ORGANIZE and LABEL your calculations clearly. No partial credit will be given without clearly labeled calculations. You do NOT have to enter the numbers into a calculator. You simply need to show the last step before punching it in.

You have a 1L of a 0.6M buffer of ahernium (pKa = 9.11) in which the concentration of acid is twice that of the salt.

a. Write an equation to calculate the pH of this buffer (4 points)

pH = 9.11 + log{[.2]/[.4]}

b. If you wish to get the buffer to maximum buffering capacity, describe whether you would use HCl or NaOH and how many moles you would use. You will need to clearly show and label your calculations to explain your answer. (16 points)

You will need 0.1 moles of NaOH. After adding this, you will have 0.3M salt and 0.3M acid

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Exam 2 Key (BB 350)

Section I: The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive eight points if you circled 'b','c', and 'd'. You would receive two points if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings. These are essentially true/fall questions. True answers are shown in RED.

Practice question #A: Oregon State University

A. is a factory in Portland
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world

1. With respect to fatty acids,

A. more unsauration lowers their melting temperatures
B. greater length raises their melting temperatures
C. oils have more unsaturated fatty acids than fats
D. fish membranes have more unsaturated fatty acids than humans

2. With respect to components found in nucleic acids,

A. adenine does not contain a sugar
B. nucleosides do not contain a sugar
C. nucleotides do not contain a phosphate
D. RNA contains ribose, but not deoxyribose

3. With respect to membranes,

A. integral membrane proteins project through both sides of the lipid bilayer
B. liposomes are human made structures
C. carbon dioxide cannot cross the lipid bilayer
D. cholesterol raises the transition temperature of a membrane

4. With respect to control of enzyme activity,

A. covalent modification always activates enzymes
B. allosterism always inactivates enzymes
C. feedback inhibition works allosterically

Section II: 1. Each sentence below in this section is missing a word or short phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded three points. You MUST be precise in your definitions.

1. What molecule found in the membranes of cells widens the cells' transition temperatures (T_m values)?

cholesterol

2. Define allosterism (no points for “being close”)

when binding of a small molecule to an enzyme affects the enzyme’s activity

3. ATCase has two different kinds of subunits. What category of subunit does aspartate bind to?

catalytic subunits

4. Carbohydrates are only found attached to the outside of cell membranes. What is one function, as described in class?

cellular identity

5. What is the driving force for the movement of molecules in facilitated diffusion?

concentration difference (diffusion)

6. What three carbon molecule forms the backbone of glycerophospholipids, but not sphingolipids?

glycerol

7. What is required for a membrane transport system to be described as ‘active transport’?

at least one molecule must be moved against a concentration gradient

8. Name an inhibitor of prostaglandin synthesis

aspirin, ibuprofen (other COX inhibitors)

9. What chemical change happens to vitamin A that results in nerve signaling?

cis-trans bond isomerization

10. What is the chemical difference between a deoxyribonucleotide and a deoxyribonucleoside (be precise in your answer)?

a deoxyribonucleoside has no phosphate, whereas a deoxyribonucleotide has at least one phosphate

11. In attenuation of the trp operon, when there is sufficient tryptophan, what process is terminated?

transcription

Section III – Problems/Long Answer

1. *E. coli* DNA polymerase has three essential activities for DNA replication. Name and explain the enzymatic activity that each one catalyzes.

5’ to 3’ DNA polymerase - makes DNA

3’ to 5’ exonuclease - proofreading - removes mispaired bases

5’ to 3’ exonuclease - removes RNA primers

2. Draw out the central dogma and identify the exception to it with respect to retroviruses, such as HIV

DNA -> RNA -> Protein

The exception of retroviruses is that they can make DNA using RNA as a template

3. I described in class how *E. coli* can switch from making one class of genes to making another class of genes, due to changes of circumstances. Describe precisely what is involved in making this switch (hint – this has nothing to do with lactose).

switching of classes of genes in *E. coli* can happen as a result of changing which sigma factor is being synthesized

4. The lac operon has controls that allow it to adapt to the cell’s needs for energy. Explain all of the controls listed in class and how they allow for this adaptation.

lac repressor - binds to lac operator when lactose absent, turning off transcription of the operon. When lactose present, it binds to the lac repressor and prevents it from binding to the lac operator, thus allowing transcription to occur

CAP - when bound to cAMP it binds near the lac operator and stimulates transcription to occur

5. List and describe three modifications to eukaryotic mRNAs that do not happen to prokaryotic mRNAs.

5’ cap

3’ poly-A tail

splicing

Exam 3 Key (BB 350)

Section I: The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive eight points if you circled 'b','c', and 'd'. You would receive two points if you circled 'a' and 'b'. You would receive no points if you circled only 'a'. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings. These are essentially true/fall questions. True answers are shown in RED.

Practice question #A: Oregon State University

A. is a factory in Portland
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world

1. With respect to tools for biotechnology,

A. a plasmid is a circular DNA that replicates in human cells
B. a polylinker is a short stretch of DNA with multiple restriction sites in a plasmid
C. enzymes used to cut DNAs at specific places are called methylases
D. a plasmid for making protein in a cell would have to have a promoter

2. With respect to sugar structures,

A. a hexose cannot be a furanose
B. the Fischer straight chain structure of glucose has no anomeric carbon
C. a reducing sugar is easily reduced
D. a glycoside always has a bond involving the anomeric carbon

3. With respect to translation,

A. the 23s rRNA catalyzes the formation of peptide bonds
B. initiation does not involve any peptide bond formation
C. EF-Tu is involved in translocation
D. ATP is required for translocation

4. With respect to polysaccharides,

A. cellulose is a polymer of glucose
B. chitin is a polymer of fructose
C. bacterial cell walls contain peptidoglycan structures with D-amino acids

Section II: 1. Each sentence below in this section is missing a word or short phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded three points, except as noted. You MUST be precise in your definitions.

1. What is the name of the bacterial protein factor responsible for translocation during translation?

EF-G

2. Precisely where is the wobble base of the anti-codon?

5’ end of anti-codon

3. Describe the physical appearance of a messenger RNA during eukaryotic translation

circularized

4. Give the sequence (5’ to 3’) of the three stop codons (1 point each)

UAA, UAG, and UGA

5. What is the precise name of the first amino acid put into bacterial proteins? (must be exact)

f-met (formyl-methionine)

6. What is the function of a chaperonin?

assist proteins in folding properly

7. What is the precise name we give to a gene that after it is mutated can cause a cancer?

proto-oncogene

8. Ras causes uncontrolled growth after it is mutated. What does the mutation in ras cause it to be unable to catalyze?

conversion of GTP to GDP

9. Name one electron carrier described in class in its oxidized form

NAD$^+$, FAD, or NADP$^+$

10. Describe how one makes a sugar alcohol

reduce a sugar

11. Describe the structural difference between amylose and amylopectin

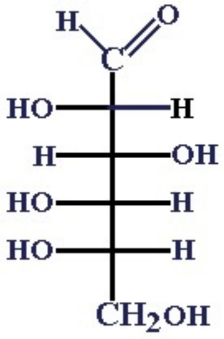
amylose is a polymer of glucose with only alpha-1-4 linkages

amylopectin is a polymer of glucose with alpha-1-4 linkages plus alpha 1-6 linkages every 30-50 residues

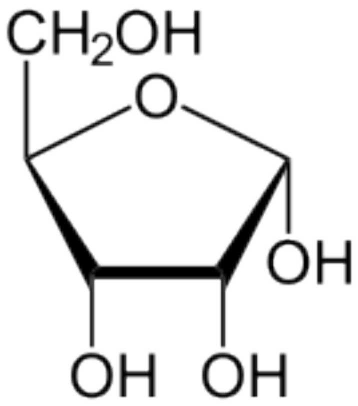
12. What animal polysaccharide is similar to amylopectin?

glycogen

13. Draw the structure of L-glucose in the Fischer form



14. Draw the structure of alpha-D-ribose as a furanose



15. What important molecule is an intermediate in the reaction catalyzed by phosphoglycerate mutase?

2,3-BPG

16. Name the animal product of fermentation

lactate

17. What pathway described in class is an important source of NADPH?

pentose phosphate pathway

Section III – Problems/Long Answer

1. The initiation of prokaryotic translation is a very intricate process in which multiple factors must come together to prepare for the elongation phase. Describe all of the factors described in class, the order in which they come together, and the final structure at the end of initiation. If you list steps in the elongation phase, you will lose points.

Elongation involves three initiation factors, IF-1, IF-2, and IF-3. These combine first with the 30S ribosomal subunit and the mRNA where the Shine-Dalgarno site of the mRNA is lined up with a complementary region of the 16S rRNA. Then the initiator tRNA (f-MET) and large subunit are brought to the system to create the intact ribosome with mRNA running through it and a single tRNA-fMET in the P-site.

2. A reaction goes as follows

Student <=> Instructor

The reaction goes forward when there are equal amounts of Student and Instructor. Similarly, the reaction goes forward when there is ten times as much Student as there is Instructor. Is it possible for the reaction to go forward when there is ten times as much Instructor as Student? If yes, you must state why so. If no, your answer must similarly answer why so. If it is impossible to answer, your answer must also state why so. Any answer you give absolutely be based mathematically in energy. Answers without a mathematical justification will receive no credit.

Yes, it is possible. If the ΔG°' value is sufficiently negative, it can counterbalance any positive values arising from the scenarios described above

3. The Cori cycle helps the body to adapt to important situations. Describe the situation(s) it helps to adapt to. In addition, show the relevant places in the body where the reactions occur and show what the products of each relevant organ of the body is. A diagram will be helpful.

Liver - absorbs lactate from bloodstream and converts it to glucose. Glucose exported to bloodstream

Muscles - absorb glucose from bloodstream and produce lactate, which they export to the bloodstream.

The process occurs during periods of exercise when the body is unable to provide sufficient oxygen to the muscles.

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Final Exam Key (BB 350)

Section I: The statements in this section can be completed by any of the lettered responses following it. Each statement may have more than one answer that is correct, one answer that is correct, or no answers that are correct. Students should clearly circle only those responses that complete the sentence to make a correct statement. Points will be awarded for each circled response that makes a correct statement and for each uncircled response that makes an incorrect statement. For example, the practice question below has three correct answers (b,c,d). You would receive four points if you circled ‘b’,’c’, and ‘d’. You would receive one point if you circled ‘a’ and ‘b’. You would receive no points if you circled only ‘a’. If we have uncertainty about whether or not an answer is marked, it will automatically be counted as a wrongly answered question. Be clear in your markings. These are essentially true/fall questions. True answers are shown in RED.

Practice question #A: Oregon State University
A. is a factory in Portland
B. is located in Corvallis, Oregon
C. has a mascot named Benny Beaver
D. has students from all over the world

1. Hydrogen bonds
A. are weaker than covalent bonds
B. occur as a result of ionization
C. are the bonds formed between cysteines
D. stabilize secondary, tertiary, and quaternary structures

2. With respect to amino acids,
A. glycine is smaller than proline
B. all of them have a pI
C. biological ones are almost always in the D configuration
D. they can be substrates for transaminases

3. With respect to membrane lipids,
A. sphingolipids contain glycerol
B. glycerophospholipids do not contain fatty acids
C. sphingolipids are common in brain tissue
D. cholesterol is common in brain tissue

4. With respect to mRNAs,
A. they carry the anti-codon
B. there are 64 of them, in theory
C. they contain start and stop codons
D. they have caps in prokaryotes

6. With respect to viruses,
A. HIV is a retrovirus
B. they sometimes carry oncogenes
C. they can use reverse transcriptase to make RNA from DNA
D. they can infect prokaryotes and eukaryotes

7. With respect to lipid metabolism
A. fats are more reduced than carbohydrates
B. fatty acid synthesis occurs in the cytoplasm
C. ACP carries fatty acids across the inner mitochondrial membrane
D. fat breakdown is stimulated by insulin

8. With respect to photosynthesis,

  A. protons are pumped outside the chloroplast
  B. oxygen is the terminal electron acceptor
  C. ATP is produced by substrate level phosphorylation
  D. oxygen is produced in the dark cycle

Section II: 1. Each sentence below in this section is missing a word or short phrase to complete it. Fill in the blank as appropriate to complete the sentence with a correct statement. Each correctly filled in blank below will be awarded three points. You MUST be precise in your definitions.

1. What does one have to remove in order to convert a weak acid into a salt?

a proton

2. Which amino acid in the catalytic triad of chymotrypsin gains a proton transiently during the catalytic cycle?

histidine

3. Explain why myoglobin does not exhibit cooperativity

it only has a single subunit

4. Define cooperativity

when binding of one molecule to a protein favors binding additional molecules of the same type by the same protein

5. How does the pK_a of a very weak acid compare to the pK_a of a weak acid ?

the pK_a of a very weak acid is higher than the pK_a of a weak acid

6. Explain precisely the piece of information that one would always know if one had the K_m for an enzyme.

the substrate concentration at $V_{max}/2$

7. Name the strongest force stabilizing tertiary structure

disulfide bonds

8. Which strand in DNA replication contains the Okazaki fragments?

lagging strand

9. What is the name of the sequence a few bases before the *E. coli* (bacterial) translational start site?

Shine-Dalgarno site

10. What enzyme present in the developing embryo ‘builds’ the ends of the linear eukaryotic chromosomes?

telomerase

11. What trait commonly associated with a plasmid allows a researcher to easily identify which cells get the desired DNA during transformation?

antibiotic resistance

12. What is the name of the enzyme that is essential for removing primers in *E. coli* DNA replication?

DNA Polymerase I

13. What protein in translation is responsible for translocation?

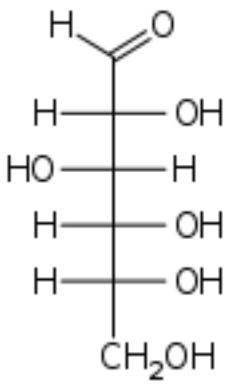
EF-G

14. What is on the 5’ end of eukaryotic mRNAs that is not on the 5’ end of prokaryotic mRNAs?

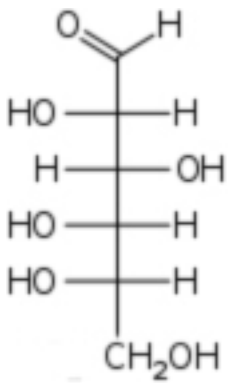
a cap structure

15. Draw the Fischer form of D-glucose and its enantiomer (6 points)

D-Glucose =



Enantiomer =



16. When cells are going through fermentation, what molecule are all of them trying to produce?

NAD^+

17. Define feedback inhibition

when the last molecule in a metabolic pathway inhibits the first enzyme in that pathway

18. Besides glycogen, what other molecule is the substrate of glycogen synthase?

UPD-glucose

19. What enzyme of gluconeogenesis is inhibited by F2,6BP?

fructose-1,6-bisphosphatase (F-1,6BPase)

20. Besides acetyl-CoA, what is the only other 2 carbon molecule in the glyoxylate cycle?

glyoxylate

21. What enzyme catalyzes the ‘big bang’ of glycolysis?

pyruvate kinase

22. Which molecule of the electron transport system is referred to as the ‘traffic cop’?

coenzyme Q (CoQ)

23. Name the molecule that directly donates two carbons to the growing fatty acid chain.

malonyl-ACP

24. What enzyme of the cholesterol biosynthesis pathway is feedback inhibited?

HMG-CoA reductase

25. Besides palmitoyl-CoA, what other molecule listed in class is a precursor of sphingolipids?

serine

26. What molecules are substrates for ribonucleotide reductase in making deoxyribonucleotides (answer must be precise)

ribonucleoside diphosphates

27. When you develop gout, what molecule is precipitating in your cells?

uric acid

28. Name an anti-cancer drug discussed in class that mimics folate

methotrexate

Section III – Problems/Long Answer

1. One liter of a buffer from the blood of Modest Mouse singer Isaac Brock is found to have a pH of 7.2. The pKa of this buffer is 7.4. Does the buffer have more salt or more acid?

more acid

Use the Henderson-Hasselbalch equation to justify your answer. Your answer MUST be based in the Henderson-Hasselbalch equation. Other answers will automatically be wrong. (7 points)

7.2 = 7.4 + log{[salt]/[acid]}

Thus -0.2 = log{[salt]/[acid]}, so there much be more acid than salt

2. Describe the sequence of events involved in the elongation of translation in prokaryotes. Name the relevant proteins, RNA molecules, and structures described in class and the functions they perform. (9 points)

Three elongation factors of which two were named in class -

EF-Tu (carries proper amino acid to A site of ribosome and checks to be sure there is proper pairing between the anticodon of the tRNA and the codon of the mRNA. Removes tRNA if pairing not right. Also protects tRNA-amino acid bond from water.)

EF-G - uses energy of hydrolysis of GTP to move ribosome one codon farther down the ribosome

Sequence - tRNA-AA bound by EF-Tu enters A site of ribosome. If codon-anticodon pairing is proper, EF-Tu cleaves GTP and leaves. Otherwise EF-Tu removes tRNA-AA from A site. When proper tRNA in A site, 23S rRNA catalyses a peptide bond between the amino acid attached to the tRNA in the P site and the amino acid on the tRNA in the A site. Newly bonded structure is left linked to the tRNA in the A site. EF-G uses energy of hydrolysis of GTP to move ribosome one codon further in the 3' direction. This leaves the tRNA-peptide structure in the P site and the A site is left open. The previous tRNA that was in the P site moves to the E site and exits the ribosome.

3. A reaction has twice as much product as reactant at equilibrium. What is the value of the ΔG°'? (I am not looking for a number here) (2 points)

$$\Delta G°' = -RT\ln\{2/1\}$$

Which direction will the reaction go when there are equal amounts of reactant and product? (Your answer MUST be based in the ΔG equation) (6 points)

The ΔG°' value above is negative. Thus when there are equal amounts of reactant and product,

ΔG = negative number + $RT\ln\{1/1\}$ = negative number + 0

Consequently , ΔG = negative number so reaction will move forward

4. Compare and contrast electron transport and oxidative phosphorylation to the light reactions of photosynthesis with respect to electron sources, electron acceptors, movement of protons, and any products of these reactions.

Photosynthesis

electron source - water

electron acceptor - NADP$^+$ (makes NADPH)

splitting electrons and protons from water generates molecular oxygen as a byproduct

protons are pumped into the thylakoid space of the chloroplast by electron movement during the light reactions. ATP is made by movement of the protons out of the thylakoid space through the ATP synthase

ETS/Oxidative phosphorylation

electron sources - NADH and FADH$_2$

electron acceptor = oxygen, making water.

NAD$^+$ and FAD are byproducts

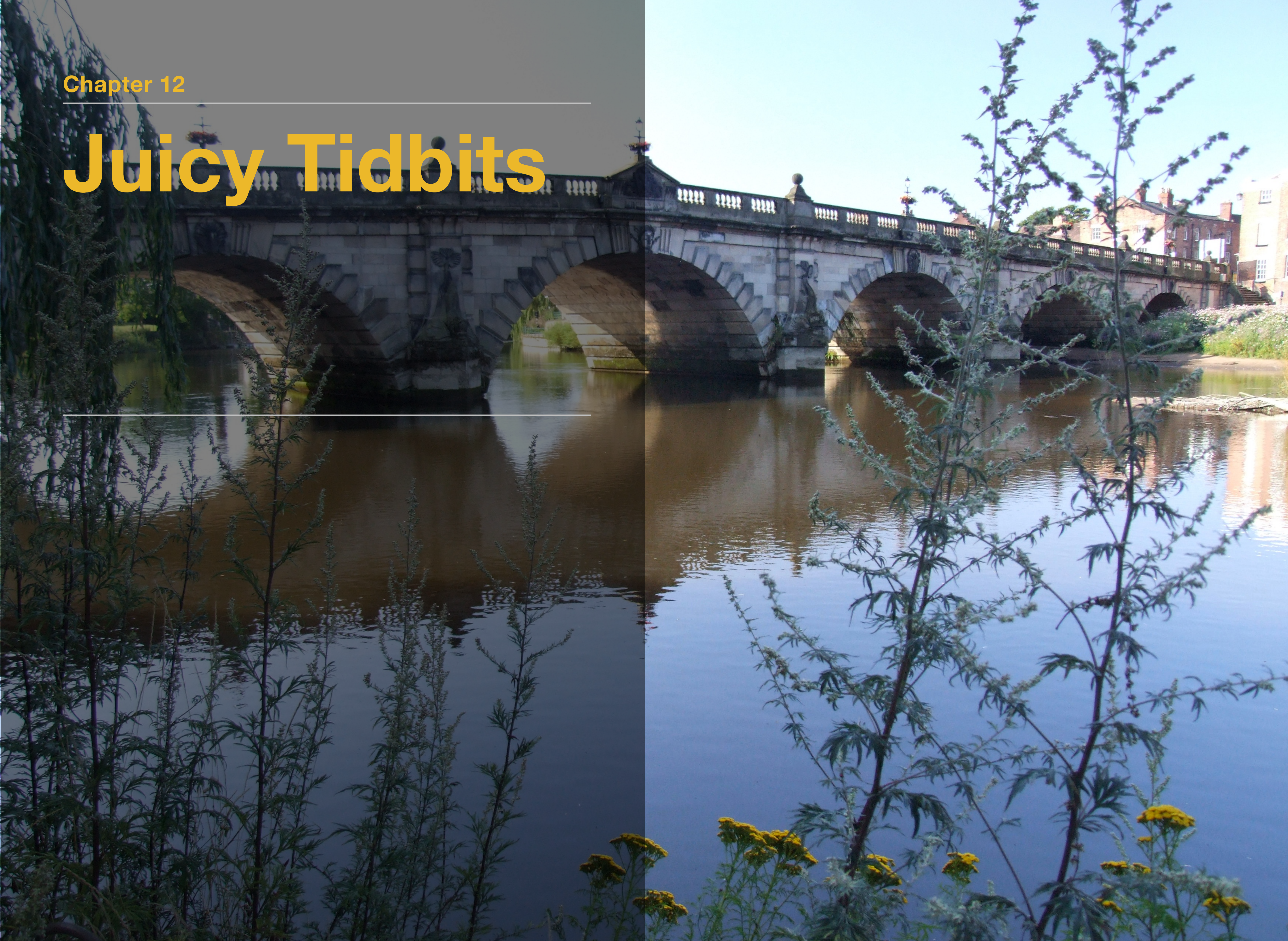
protons are pumped out of the mitochondrial matrix to the intermembrane space of the mitochondrion by electron movement during electron transport. ATP is made in oxidative phosphorylation by movement of the protons back into the mitochondrial matrix through the ATP synthase (Complex V).

Jump to Chapter

1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9 / 10 / 11 / 12

Chapter 12

Juicy Tidbits



Juicy Tidbits

Miscellany

Due to space restrictions, we weren't able to include several songs adjacent to the relevant text and we didn't want to leave them out entirely, so we include them here starting on the next page for your enjoyment.

One last plug :-)

We appreciate your support (financial or otherwise) to say thanks for our efforts. They include buying a calendar (HERE), buying "***The Splendidest Ever Metabolic Melodies***" songbook of lyrics (HERE), registering for our ecampus classes at Oregon State University (HERE, HERE, HERE, and HERE), and/or sending us a message telling us how you've used the book. You can also join and **LIKE** our Facebook page for ***Biochemistry Free and Easy*** HERE.

Our email addresses are

kevin@davincipress.com

indira@davincipress.com

You can view our biochemistry courses as follows below:

BB 350 - HERE / Download syllabus HERE
BB 450/550 - HERE / Download syllabus HERE
BB 451/551 - HERE / Download syllabus HERE
BB 100 - HERE / Download syllabus HERE
We have a Web site full of **Metabolic Melodies** and **Limericks** that you may enjoy. The URL is

http://www.davincipress.com/metabmelodies.html

Kevin released a book of **Limericks** in early 2013. More information about that HERE. He also a few YouTube music videos he is proud of. They include an anti-smoking video called "***I Lost a Lung (from smoking Camels)***" and is to the tune of "*I Left My Heart in San Francisco*" The link is HERE. The Oregon rain gets noted in "***Let It Rain***" (to the tune of "*Let It Snow*") HERE, "***Around the Nucleus***" (to the tune of "*Across the Universe*") is HERE, and the ***OSU Alma Mater*** (to the tune of "*Carry Me Back to OSU*") HERE.

Ode to Bicarbonate

To the tune of "*Song Sung Blue*"

H-2-O
Ionizes slowly
You should know
Its behavior wholly

It presides - in cells' insides - a solvent great
But mix it with an oil and shake it up
You'll see 'em separate
See 'em separate

C-O-2
Made in oxidation
Inside you
Decarboxylation

So divine – when it combines up with the H-2-Os
Bonding together makes bicarbonates
To ease the pH woes

Bicarbonate – can conjugate and take protons out
Restoring a balance inside of the blood
Of this there is no doubt

Thus it's so
Thanks to bicarb buffer
In blood flow
You don't have to suffer

Bicarbonate – will conjugate to take the protons out
Restoring a balance inside of the blood
Of this there is no doubt

Protons stow
In the bicarb buffer
Never grow
So you do not suffer
(fade)

Recorded by David Simmons
Lyrics by Kevin Ahern

Translation

To the tune of "*Maria*" - from West Side Story

Translation
The most intricate thing I ever saw
From five prime to three prime, translation, translation

The final step that we know about the central dog-ma
Amino, carboxyl, translation, translation. . . .
Translation, translation, translation . .

Translation!
I just learned the steps of translation
And all the things they say
About tRNA
Are true

Translation!
To form peptide bonds in translation
The ribosomal cleft
Must bind to an E-F
tee-you!

Translation!
A-U-G binds the f-met's cargo
16S lines up Shine and Dalgarno
Translation
I'll never stop needing translation
The most intricate thing I ever saw
Translationnnnnnnnnnnnnnnnn

Recorded by Tim Karplus
Lyrics by Kevin Ahern

Chromatin

To the tune of "*Sunshine on My Shoulders*"

Charges of the lysines bind to phosphates
Minus charges cling to plusses tight
Chromatin assembly is essential
Eukaryotic cells must get it right

Cells are tiny micro-scaled enclosures
With nuclei tucked deep in their insides
That's the place amazingly enough that
Seven feet of DNA resides

H2a and H2b have lysines
That's how they get charges don't forget
Paired with H's three and four they make up
A chromatin core's octameric set

These get organized in higher orders
Changing with the cycles of the cell
Denser packing going through mitosis
At other times the structures simply swell

So because of all the hyperpacking
Nuclei can hold entire genomes
Thank the histones spooling DNA for
Physically downsizing chromosomes

Recorded by David Simmons
Lyrics by Kevin Ahern

Good Protein Synthesis

To the tune of "*Good King Wenceslaus*"

Amino acids cannot join
By themselves together
They require ribosomes
To create the tether

All the protein chains get made
'Cording to instruction
Carried by m-R-N-A
In peptide bond construction

Small subunit starts it all
With initiation
Pairing up two RNAs
At the docking station

Shine Dalgarno's complement
In the 16 esses
Lines the A-U-G up so
Synthesis commences

Elongation happens in
Ribosomic insides
Where rRNA creates
Bonds for polypeptides

These depart the ribosome
Passing right straight through it
In the tiny channels there
Of the large subunit

Finally when the sequence of
One of the stop codons
Parks itself in the A site
Synthesis can't go on

P-site RNA lets go
Of what it was holding
So the polypeptide can
Get on with its folding

Recorded by David Simmons
Lyrics by Kevin Ahern

Histones

To the tune of "*Meet the Flintstones*"

Histones, tiny histones
Wrap up eukaryotic DNA

Using lysine side chains
They arrange a chromatin array

With them - DNAs of seven feet
Fit in - side the nucleus so sweet

When you use the histones
You have to deal with condensation
And its ablation
Inside your chromosomes

Recorded by David Simmons
Lyrics by Kevin Ahern

Transcription

To the tune of "*Frosty the Snowman*"

Phos-pho-di-esters
Are the bonds of RNA
That support a ribopolymer
Made of G,C,U and A

The RNA polymerase
Binds to a Pribnow box
And copies from the template strand
All the along the way it walks

IN-i-ti-a-tion
Of transcription thus proceeds
From the closed to open complexing
In the DNA it reads

The sigma factor gets released
Its work is over fast
Polymerase can then advance
After this step has been passed

In elongation
The polymerizing spree
Moves along the way in fits and starts
Synthesizing five to three

The RNA is floppy and
It dangles from one end
Oh that's a most important thing
For you to comprehend

Then termination
Fin-ish-ES the RNAs
Thanks to protein rho or hairpin forms
That release polymerase

So this describes transcription's steps
In three part harmonies
Here's hoping with this melody
You (can) learn it all with ease

Recorded by David Simmons
Lyrics by Kevin Ahern

Structural Lullaby

To the tune of "*Brahms' Lullaby*"

In your sleep
You can keep
Learning more about sugars
Fischer schemes
Haworth rings
D & L and everything

Hydroxides
Can't collide
Fav'ring chair over boat form
Spatial guides
Coincide
With the way structures form

Recorded by David Simmons
Lyrics by Kevin Ahern

The Bloody Things

To the tune of "*Coke ® It's The Real Thing*"

I'm gonna put some oxygens
beside my porphyrin rings
To nudge the irons up a notch
and yank on histidines
The globins' shapes will change a bit,
oh what a sight to see
The way they bind to oxygen
co-op-er-AH-tive-ly

And as I exit from the lungs
to swim in the bloodstream
Metabolizing cells they all
express their needs to me
To them I give up oxygen
and change from R to T
While my amines, they hang onto
the protons readily

But that's not all the tricks I know,
there's more that's up my sleeve
Like gaps between sub-U-nits that
hold 2,3-BPG
When near metabolizing cells,
I bind things that diffuse
The protons and bicarbonates
from lowly cee oh twos

CHORUS
That's the way it is
When your cells are at play
Go say hip hip hooray
For the bloody things

Recording by David Simmons
Lyrics by Kevin Ahern

The New Serine Protease Song

To the tune of "*Rudolph the Red-Nosed Reindeer*"

You know threonine, cysteines and trusty aspartates
Cobalts, magnesium and glutamates
But do you recall
The best characterized enzymes of all?

All serine proteases
Work almost identically
Using amino acid
Triads catalytically

First they bind peptide substrates
Holding onto them so tight
Changing their structure when they
Get them in the S1 site

Then there are electron shifts
At the active site
Serine gives up its proton
As the RE-ac-tion goes on

Next the alkoxide ion
Being so electron rich
Grabs peptide's carbonyl group
Breaks its bond without a hitch

So one piece is bound to it
The other gets set free
Water has to act next to
Let the final fragment loose

Then it's back where it started
Waiting for a peptide chain
That it can bind itself to
Go and start all o'er again

Recorded by David Simmons
Lyrics by Kevin Ahern

My Old Enzymes

To the tune of "*Auld Lang Syne*"

Whene'er my proteins go kaput
If they are past their prime.
The cells will act to soon replace
All of my old enzymes

They know which ones to break apart
Ubiquitin's the sign
A marker for pro-TE-a-somes
To find the old enzymes

These soon get bound and then cut up
In pieces less than nine
More chopping yields the single ones
Building blocks from old enzymes

So in a way the cell knows well
Of father time it's true
Amino acids when reused
Turn the old enzymes to new

Recorded by David Simmons
Lyrics by Kevin Ahern

The End

In Closing . . . .

The End

To the tune of "*The End*"

Hoc est finis

This is the end

Of Free and Easy Biiiiiii-o-chem

Recorded by David Simmons
Lyrics by Kevin Ahern

Copyright Information

& Disclaimer

Biochemistry Free and Easy ©2013 Kevin Ahern & Indira Rajagopal

All rights reserved

This is version 2.0 of this electronic book. It is also version 2.0 of the PDF form of this book.

Disclaimer

Every effort was made to ensure that information contained in this publication was as accurate as possible at the time of publication (July 24, 2012). However, Kevin Ahern and Indira Rajagopal make no claims that the information contained anywhere in this publication is, in fact correct, so users assume responsibility for all ways in which they use the information herein. This publication is therefore provided as is and all responsibility for use of information herein resides solely on the user. Further, Kevin Ahern and Indira Rajagopal make no claims about medical validity and offer no medical advice regarding anything stated in this books nor to hyperlinks to any other content provided here. Kevin Ahern and Indira Rajagopal offer no advice of any sort. Anyone seeking medical or other advice needs to consult medical or other relevant professionals for such advice.

Wikipedia Licenses

Figures from Wikipedia were used under the Creative Commons or GNU license and are listed according to their URLs as follows.

Page 13 – Onion cells - http://en.wikipedia.org/wiki/File:Wilson1900Fig2.jpg

Page 14 – Extremophile - http://en.wikipedia.org/wiki/File:Grand_prismatic_spring.jpg

Page 14 – Water/Iceberg - http://en.wikipedia.org/wiki/File:Iceberg_with_hole_near_sanderson_hope_2007-07-28_2.jpg

Page 36 – Light reactions photosynthesis - http://en.wikipedia.org/wiki/File:Z-scheme.png

Page 43 – Amino acid - http://en.wikipedia.org/wiki/File:AminoAcidball.svg

Page 45 – Protein structure - http://en.wikipedia.org/wiki/File:Main_protein_structure_levels_en.svg

Page 46 – Beta sheet - http://en.wikipedia.org/wiki/File:Beta-meander1.png

Page 46 – Tropocollagen - http://en.wikipedia.org/wiki/File:Collagentriplehelix.png

Page 48 – Ramachandran plot http://en.wikipedia.org/wiki/File:Protein_backbone_PhiPsiOmega_drawing.jpg

Page 48 – Myoglobin - http://en.wikipedia.org/wiki/File:Myoglobin-1mba.png

Page 50 – Water on leaf - http://en.wikipedia.org/wiki/File:Water_drop_on_a_leaf.jpg

Page 57 – Prions - http://en.wikipedia.org/wiki/File:Prion_propagation.svg

Page 60 – A,B, Z DNA - http://en.wikipedia.org/wiki/File:A-DNA,_B-DNA_and_Z-DNA.png

Page 61 – Supercoiling - http://en.wikipedia.org/wiki/File:Circular_DNA_Supercoiling.png

Page 63 – tRNA - http://en.wikipedia.org/wiki/File:TRNA-Phe_yeast_1ehz.png

Page 68 – Oligosaccharide - http://en.wikipedia.org/wiki/File:Branched_oligosaccharide_struct.svg

Page 70 – Glycogen - http://en.wikipedia.org/wiki/File:Glykogen.svg

Page 70 – Cellulose - http://en.wikipedia.org/wiki/File:Cellulose_Sessel.svg

Page 70 – Glycosaminoglycan - http://en.wikipedia.org/wiki/File:Hyaluronan.png

Page 79 - Vitamin D - http://en.wikipedia.org/wiki/File:Cholecalciferol-3d.png

Page 99 - Ribozyme - http://en.wikipedia.org/wiki/File:Ribozyme.jpg

Page 108: Proofreading - http://commons.wikimedia.org/wiki/File:DNA_polymerase.svg

Page 119: T7 RNA polymerase - http://commons.wikimedia.org/wiki/File:T7_RNA_polymerase_at_work.png

Page 144 - Glycolysis - http://commons.wikimedia.org/wiki/File:Glycolysis.svg

Page 182 - Calvin Cycle - http://en.wikipedia.org/wiki/File:Calvin-cycle4.svg

Page 189 - Trp metabolism - http://en.wikipedia.org/wiki/File:Tryptophan_metabolism.png

Page 199 - Nerve junction - http://commons.wikimedia.org/wiki/File:Neuron_synapse.png

Page 205 G-protein pathway - http://commons.wikimedia.org/wiki/File:G-protein.svg

Page 207 PKC activation - http://commons.wikimedia.org/wiki/File:Activation_protein_kinase_C.svg

Page 218 - DNA fragment separation - http://commons.wikimedia.org/wiki/File:Gel_electrophoresis_2.jpg

Page 219 - SDS-PAGE separation of proteins - http://en.wikipedia.org/wiki/File:SDS-PAGE.jpg

The End

To the tune of "*The End*"

Hoc est finis

This is the end

Of Free and Easy Biiiiiii-o-chem

Recorded by David Simmons
Lyrics by Kevin Ahern

ΔG

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

1,3 BPG

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

1,3-bisphosphoglycerate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

1/V vs. 1/[S

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

2-PG

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

2,3 BPG

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

3-PG

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

3-phosphoglycerate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

6-phosphogluconate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

7-dehydrocholesterol

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Acetoacetate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Acetoacetyl-CoA

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Acetyl-CoA

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Acetyl-CoA carboxylase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Acetylcarnitine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Aconitase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

ACP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Activation energy

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Active site

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Acyl-carrier protein

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Acyl-CoA dehydrogenase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Adenine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Alanine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Aldose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Alkoxide ion

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Allosterism

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Allostery

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Alpha helix

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Alpha keratin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Alpha-helix

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Alpha-keto-glutarate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Alpha-ketoglutarate dehydrogenase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Amino acids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

AMP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Amphiphilic

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Amylopectin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Amylose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Anabolic

Lorem ipsum dolor sit amet, consectetur adipisicing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisi ut aliquip ex ea commodo consequat.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Anabolic processes

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Anomeric carbon

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Arachidonic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Arginine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Argninosuccinate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Ascorbic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Asparaginase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Asparagine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Aspartate transcarbamoylase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Aspartic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Aspirin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

ATCase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

ATP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Bends

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Beta oxidation

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Beta sheets

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Beta strands

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Beta-carotene

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Beta-hydroxybutyrate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Beta-keratin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Bile acids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Biochemistry

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Boat

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

C4 plants

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Calvin Cycle

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Carbamoyl phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Carbamoyl-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Carbohydrates

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Carbonic anhydrase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Carnitine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Catabolic processes

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Catalysis

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index ☐ Find Term

Chapter 4 - Catalysis

Catalytic triad

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Cellulase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Cellulose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Chair

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Chenodeoxycholic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Chitin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Chlorophyll

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Cholesterol

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Cholic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Chondroitin sulfate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Chymotrypsin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Citric acid cycle

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Citrulline

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

CoASH

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Coenzyme Q

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Coenzymes

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Collagen

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Competitive inhibition

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Complex carbohydrates

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Cooperativity

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Covalent modification

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

CTP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

CTP synthase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Cyclooxygenase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Cysteine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

D-D transpeptidase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

D-Erythrose

Index Find Term

Chapter 4 - Catalysis

D-Erythrose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

D-D transpeptidase

Index

Find Term

Chapter 3 - Structure and Function

D-Threose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Deoxycholic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Deoxyribonucleotide

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Desaturases

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

DHAP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

DHFR

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Diacylglycerol

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Dihydrofolate reductase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Dimethylallylpyrophosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Disaccharides

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Dopamine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Elongases

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Enantiomers

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Entropy

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Enzyme-substrate (ES) complex

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Enzymes

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Epinephrine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Erythrose-4-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Essential amino acids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Essential fatty acids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Ethanol

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

F1,6BP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index ☐ Find Term

Chapter 6 - Metabolism I

F1,6BPase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

F2,6BP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

F6P

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

FAD

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

FADH2

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Farnesyl-pyrophosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Fats

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Fatty Acid Synthase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Fatty acids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Feedback inhibition

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Feedforward activation

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Fermentation

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Fibrous protein

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Folate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Folding

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Francis Crick

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Fructose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Fructose-1,6-bisphosphatase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Fructose-1,6-bisphosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Fructose-2,6-bisphosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Fructose-6-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Fumarase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Fumarate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

G3P

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

G6P

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

G6Pase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Galactose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Geranyl-pyrophosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Globular proteins

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Glucogenic

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Gluconeogenesis

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Chapter 6 - Metabolism I

Glucose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Glucose-1-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Glucose-6-phosphatase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Glossary-6-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Glucose-6-phosphate dehydrogenase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Glutamic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Glutamine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Glyceraldehyde-3-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Glyceraldehyde-3-phosphate dehydrogenase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Glycerol

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Glycerol-3-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Glycerophospholipids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Glycine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Glycocholic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Glycogen

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Glycolysis

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Glycosaminoglycans

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Glycoside

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Glyoxylate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Glyoxylate cycle

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Glyoxylate pathway

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

GMP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

GTP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Guanine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Haworth structures

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Hemoglobin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Heparin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Herman Branson

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Hexokinase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Hexoses

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Histamine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Histidine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

HMG-CoA

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

HMG-CoA reductase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Hormone sensitive triacylglycerol lipase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Hyaluronic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Hydrophilic

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Hydrophilic side chains

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Hydrophobic

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Hydrophobic amino acids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Hydrophobic effect

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Hydrophobicity

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Hydroxy-Methyl-Glutaryl-CoA

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Ibuprofen

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

IMP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Inosine monophosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Insulin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Isocitrate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Isocitrate lyase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Isoleucine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Isopentenyl-pyrophosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Isoprene

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Isoprenoid pathway

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Isoprenoids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

James Watson

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Kcat

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Keratan sulfate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Ketogenic

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Ketose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

KM

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Koshland’s Induced Fit Model of Catalysis

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Lactate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Lactate dehydrogenase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Lactose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Leucine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Lineweaver-Burk

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Linoleic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Linolenic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Linus Pauling

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Lipoic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Lycopene

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Lysine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Malate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Malate aspartate shuttle

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Malate synthase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Malonyl-ACP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Malonyl-CoA

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Metabolic pathways

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Methionine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Methotrexate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Mevalonate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Monosaccharides

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Mucopolysaccharides

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

N-linked sugars

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

NAD

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

NAD+

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

NADH

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

NADP+

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index ☐ Find Term

Chapter 6 - Metabolism I

NADPH

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

NDPK

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Nitric oxide

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Non-competitive inhibition

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Norepinephrine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

NSAIDS

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Nucleic acids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Nucleophilic attack

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Nucleoside diphosphokinase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Nucleoside monophosphate kinases

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index ☐ Find Term

Chapter 7 - Metabolism II

Nucleotides

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

O-linked sugars

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Oleic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Oligosaccharide

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

OMP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Ornithine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Orotic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Oxaloacetate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Oxidative phosphorylation

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index ☐ Find Term

Chapter 6 - Metabolism I

Palmitoyl-CoA

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Penicillin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Pentose Phosphate Pathway

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

PEP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

PEPCK

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Peptide bonds

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Peptido-glycan complex

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Perfect enzyme

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

PFK

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Phenylalanine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Phi

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Phosphatases

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Phosphatidic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Phosphodiester bonds

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Phosphoenolpyyruvate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Phosphofructokinase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Phosphoglycerate mutase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Phosphoribosylpyrophosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Phosphoribulokinase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Phosphorylases

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Phosphorylation

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Pitch

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Polypeptides

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Polysaccharides

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Porin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Porphyrin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Post-translational modifications

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Primary structure

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Proline

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Propionyl-CoA

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Prostaglandin synthase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Prostaglandins

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Protease

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Protein folding

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Proteins

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

PRPP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index ☐ Find Term

Chapter 7 - Metabolism II

PRPP amidotransferase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

PRPP synthetase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Psi

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Pyrophosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Pyruvate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Chapter 6 - Metabolism I

Pyruvate carboxylase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Pyruvate dehydrogenase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Pyruvate kinase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Quaternary structure

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Ramachandran

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Reciprocal regulation

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Reducing sugars

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Repeat

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Riboflavin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Ribonucleotide

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Ribose-5-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Ribulose-1,5-bisphosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Ribulose-5-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Ribulose-5-phosphate isomerase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Rise

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Robert Corey

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Rosalind Franklin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

rRNAs

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

RUBISCO

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

S-Adenosyl-Methionine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

S1 pocket

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Scleroprotein

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Secondary

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Secondary structures

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Sedoheptulose-1,7bisphosphatase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Sedoheptulose-7-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Serine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Serine proteases

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Serotonin

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Sphingolipids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Squalene

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Steric hindrance

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Steroid hormones

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Substrate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Substrate level phosphorylation

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Succinate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Succinate dehydrogenase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Succinyl-CoA

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Succinyl-CoA synthetase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Sucrose

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Taurocholic acid

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Terpenoids

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Tertiary

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Tertiary structure

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

Thiamine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Thioester

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Thiolase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Threonine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Topoisomerases

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 3 - Structure and Function

TPP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Transaldolase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Transaminases

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Transamination

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Transketolase

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

tRNAs

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Tryptophan

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Tyrosine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

UDP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

UMP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Uncompetitive inhibition

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Urea

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

Urea cycle

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

UTP

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 7 - Metabolism II

V vs. [S]

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 4 - Catalysis

Valine

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Vitamin A

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index Find Term

Chapter 6 - Metabolism I

Vitamin C

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Vitamin D

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 6 - Metabolism I

Vitamins

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 3 - Structure and Function

Vmax

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 4 - Catalysis

Xylulose-5-phosphate

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term

Chapter 7 - Metabolism II

Zymogen

The glossary is not enabled in this version of Biochemistry Free and Easy.

Related Glossary Terms

Drag related terms here

Index

Find Term